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EFFECTS OF SULFURIC ACID AEROSOLS ON VEGETATION

by

David S. Lang
Department of Plant Pathology
University of Minnesota
St. Paul, Minnesota 55108

Submitted by
S. V. Krupa
Principal Investigator
Air Pollution Laboratory
University of Minnesota
St. Paul, Minnesota

Grant No. R-804291

Project Officer

J. H. B. Garner
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, N.C. 27711

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

New technologies for controlling emissions of pollutants to the atmosphere are always a welcome advance in the pursuit of a cleaner environment through research. A thorough study of these new technologies is in order, however, to assure that the net effect on public health is beneficial. The Catalyst Research Program, in its investigation of the automotive oxidation catalyst, provides a sound base upon which the EPA can make a responsible assessment of the effect on public health of this advanced emission control technology.

The following report assesses the effects of sulfuric acid aerosols as generated by the catalysts on vegetation.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

A continuous flow system for exposing plants to submicron aerosols of sulfuric acid has been developed and an operational model has been constructed. Exposure chambers have been designed to allow simultaneous exposures of the same plant to aerosol and control environments. All surfaces within the exposure system are composed of either Teflon or stainless steel to minimize corrosion. Submicron acid aerosols are mechanically generated and are distributed in size representative of resident particulates found in the atmosphere. Plants have been found to be injured by exposures to high concentrations of sulfuric acid aerosol (100-200 mg/m^3) for short times of 4-16 hours. Injury to vegetation caused by sulfuric acid aerosol is similar to that caused by gaseous fluoride and is characterized by marginal and tip necrosis of foliage. This injury is distinctly different from that which has been attributed to acidic precipitation. Different plant species vary greatly in sensitivity to sulfuric acid aerosol and injury to sensitive species appears to be conditioned by biological as well as physical factors. Results indicate that foliar sulfur accumulation during exposure to sulfuric acid aerosol may be subject to substantial temporal effects. The concentrations of sulfuric acid aerosol required to produce acute vegetation effects are several orders of magnitude higher than those which have been reported for catalytic emissions from automobiles. It is, therefore, unlikely that this new source of atmospheric pollution will cause any significant acute injury to vegetation. Potential problems associated with chronic injury to vegetation from atmospheric sulfates remain unresolved.

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INTRODUCTION

Within the past 25 years much research has been directed toward the study of abiotic diseases of plants. A substantial portion of this research has been specifically concerned with air pollution and its effect on vegetation. Although vegetation injury associated with exposure to air pollution has been known since the nineteenth century, carefully conducted studies which relate plant response to defined experimental conditions have been relatively recent innovations (20).

Scientists have discovered that plants are generally more sensitive than animals to air pollution injury. It has also been well documented that environmental conditions prior to, during, and after exposure play an important, if not a critical role in determining air pollutant injury to plants (31). Individual plant species have been found to show significant variations in their sensitivity to air pollutant injury, suggesting the possibility for genetic selections tolerant to pollutant stress (31).

Using the information gathered in recent years governmental regulatory agencies have been able to establish standards for pollutant doses which will cause injury to vegetation and develop appropriate control policies.

Specific air pollutants vary greatly in their relative effect on vegetation. Relative effect of a particular pollutant is a

function of three major parameters: chemical composition, concentration, and duration of exposure. Of these, chemical composition and concentration relations are well defined for many specific pollutants. Duration of exposure and its relative contribution related to varied concentrations of individual pollutants is also well known for short term or acute exposures of most major pollutants (17). However, relatively little is known concerning the effect of complex pollutant mixtures which regularly occur at low concentrations for extended periods of time in the ambient environment (chronic exposures) (23).

As indicated, much work remains to be done in elucidating traditional pollutant-plant interactions. Additionally, continual revaluation and redefinition of previous studies relating to biological effects is necessary to incorporate technological advancements in pollutant characterization. Such a need has arisen from recent advancements made in the understanding of atmospheric aerosols and their relative contribution to total pollution effects on ecosystems (9).

The studies described in this thesis relate to a particular form of aerosol pollution resulting from the oxidation of elemental sulfur in fossil fuels during combustion processes. This research was sponsored by a grant from the United States Environmental Protection Agency Catalyst Research Program. Research sponsored under this program was specifically concerned with the characterization and definition of biological effects of sulfuric acid aerosol emissions from automobiles equipped with catalytic converters (18). It has been known for some time that elemental sulfur in fuel is oxidized relatively more efficiently in catalyst equipped cars (11). The

SO_3 ultimately formed reacts immediately with ambient water vapor to form fine particles of concentrated sulfuric acid (6,30,33). Sulfuric acid aerosol is highly reactive and may be neutralized by ammonia or combine with metallic oxides to form salts (Charleston). It is also produced via photo-oxidation of SO_2 in the presence of oxygen and nitrogen. This aerosol ranges in size from a few thousandths of a micron to about 1 micron in diameter (32). Most of these aerosols are respirable by animals and may be easily incorporated in the gaseous exchange processes of vegetation. The purpose of this study was to define the relative effect of sulfuric acid aerosols on vegetation.

Previous reports of laboratory and field studies on particulate-induced vegetation injury have been primarily limited to the exposure of plants to very large particles (13). Most of these studies have dealt with specific kinds of dusts as they pertain to local field problems (24). Few studies have been undertaken to determine the effects of acid mists on vegetation. Where such studies have been reported, the symptoms produced have resembled those expected if small droplets of sulfuric acid were deposited on the leaf surfaces, i.e., small necrotic pits scattered more or less uniformly over the leaf surface rather than in specific patterns as are caused by sulfur dioxide (8,26). According to Thomas (29) when leaves were exposed to acid mist no injury occurred if the particles were smaller than 1 micron in diameter. Larger particles settled on the leaf but did not wet dry surfaces. It was only when the leaf surface was wet and the droplets spread out that characteristic markings were developed.

Thomas (29) stated that injury due to sulfuric acid mist has never been observed in the field. However, Middleton et al. (20) reported symptoms, which resembled those caused by acid mist, on vegetation in the Los Angeles area.

Recent work in aerosol physics and atmospheric chemistry has cast considerable doubt upon the credibility of past studies, (which did not consider the specific effect of particle size). Atmospheric particulates have been shown to exist in three modes ranging from .015 to 30 micron particle size (34). Most particulate sulfate is thought to be associated with the accumulation mode (0.15 to 0.5 microns) since the other modes exist only transient species (34). Questions concerning fine particulate sulfates have not been considered in previous studies relating to vegetation effects and have only recently (largely after 1970) been studied in relation to animal effects.

Two objectives have been addressed in this study: (1) to design and construct a system for exposing vegetation to fine acid aerosols and (2) to define the effects of submicron sulfuric acid aerosols on several diverse species of vegetation.

The acid aerosol-vegetation exposure system was designed to meet two important criteria: (1) simultaneous exposure of different branches of the same plant to aerosol and control environments and (2) generation and distribution of submicron sulfuric acid aerosol within a closed exposure system essentially inert to the corrosive effects of concentrated sulfuric acid. Simultaneous exposure of the same plant to aerosol and control environments is an important criterion when

one considers the significant variation in injury which can occur between plants of the same species in response to pollutant stress (31). In order that plant response in these controlled exposures be comparable to the ambient environment it was necessary to insure that a large amount of the aerosol produced be submicron in size.

We feel the approaches described here have the facility of being applied toward the study of general aerosol problems. Altshuller (1) and others have reported on the origin and transport of sulfate aerosols and it is likely that the effects of long term chemical loading of remote environments by atmospheric pollutants will become an area of significant concern in the future. Whitby and others have studied long distance pollutant transport mechanisms and conclude that much of the transport occurs in the form of aerosol ranging in size from 0.01 to 2 μm in diameter (34). Ultimately, the aerosol is carried to the earth's surface by wet and dry deposition processes. It is hoped that the application of the exposure system developed in the present study could prove valuable in many studies relating to the effects of aerosols on vegetation.

SECTION I

DESIGN AND CONSTRUCTION OF A SUBMICRON ACID AEROSOL EXPOSURE SYSTEM FOR VEGETATION

1. GLASS HOUSE ENCLOSURE

1.1 Design and Construction

An aerosol exposure facility was developed in a 40 m^2 enclosed space of a greenhouse. This area was located in the northwest corner to allow natural lighting from the west, north and top. The south and east faces were isolated from the rest of the greenhouse by constructing a temporary wall of 5 cm by 10 cm wood supports spaced on .6 m centers with 7.6 cm thick styrofoam panels. This design served to minimize solar radiation loading during the summer months, allow independent heating and cooling, and isolate the area from normal greenhouse particulate contaminants, insects and biotic pathogens. The principal advantages of this location were the opportunity to use natural photoperiods and provision of similar environments for plant growth and exposure. The completed facility provided housing for two greenhouse benches, six exposure chambers, space for instrument storage and counter top work surface. Figure 1 shows the layout of this facility.

1.2 Heating and Cooling

Specific heating and cooling capacity was found to be necessary to minimize daily cyclic temperature and humidity variation. Separate

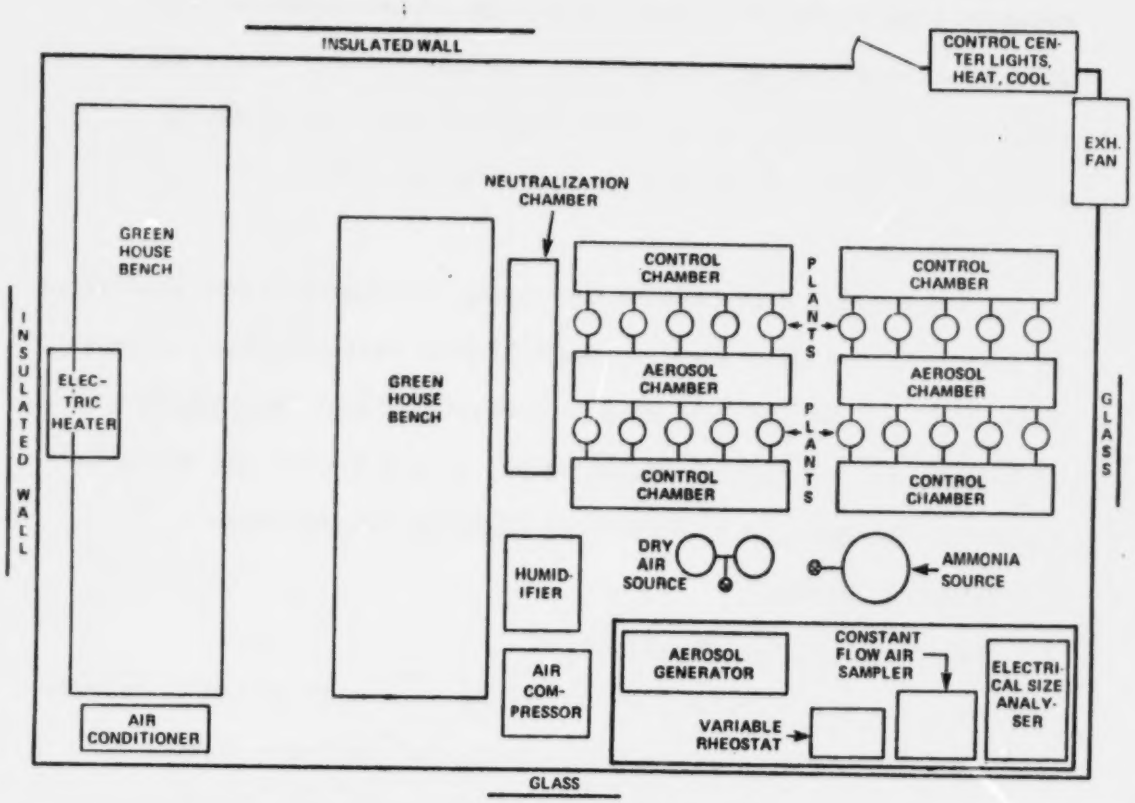


Figure 1. Layout of greenhouse facility for exposing plants to sulfuric acid aerosol.

steam heat controls, and a 4284 kg-cal electric heater, coupled with 6300 kg-cal air conditioner controlled by a centrally located thermostat substantially reduced temperature and humidity variations. Figure 2 shows optimal temperature and relative humidity control achieved during fall and winter months. It was not possible to maintain this degree of control during the summer months on days when solar heat loading exceeded the cooling capacity of the air conditioner. However, it was possible under most conditions to maintain the daily temperature variation within $\pm 2°$ C.

1.3 Lighting

Fluorescent and ultraviolet enhancing fluorescent lamps controlled by a timer switch maintained standard 16-hour photoperiods. Although some plant species appeared to grow normally without supplementary lighting, bean and soybean showed marked etiolation for all but a few months of the year unless additional lighting was provided.

2. EXPOSURE CHAMBERS

2.1 Chamber Design and Construction

Original plans called for an all-glass exposure system to provide an inert environment which would minimize the corrosive effects of H_2SO_4 and potential release of unknown substances which might have complicated the assessment of vegetation effects. However, there were some objections to the use of glass:

1) Cost increases involved made the system prohibitive
2) Delivery times on special order glass would have prevented meeting the proposed schedule of investigation
3) One of the external reviewers of our research proposal

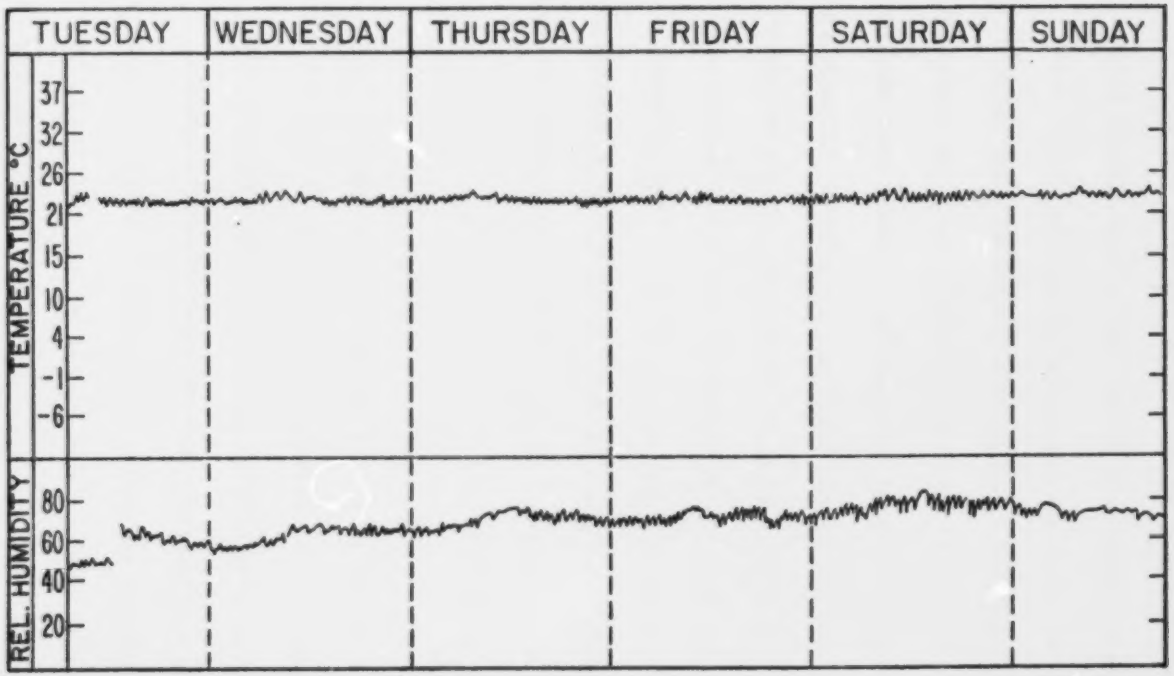


Figure 2. Temperature and relative humidity record showing optimal environmental control in greenhouse exposure facility.

suggested that Teflon be used instead of glass.

For these reasons we designed a system consisting of clear acrylic plastic support tubes 0.30 meters in diameter and 1.20 meters in length. The interior was lined with clear Teflon film (0.076 mm thick) held in place by circumferential stainless steel bands placed 0.20 meters apart through the length of each tube. Each tube was fitted with machined Teflon ports 6.4 cm in diameter through which plant branches could be inserted. Ports were machined to match the internal radius of the tube and to project through the tube. This provided a tight fitting at each port. Expanded polyethylene foam plugs were slit to accommodate the plant branch and covered with Teflon film on the interior surface. This allowed branches to be "plugged" into the exposure chamber and maintained a secure fitting between the plant and the chamber. A single exposure chamber system is shown in Figure 3.

2.2 Operation

Figures 4A and B show several chambers arranged for vegetation exposure. This system was efficient in that it allowed two opposite branches of the same plant to be simultaneously but separately exposed to the aerosol and the control environments. In operation, each chamber was maintained under a slightly negative pressure (.25 cm HOH) to prevent the escape of aerosol into the ambient air. An exhaust blower was connected via 5 cm diameter tubing to each chamber. Exhaust aerosol was passed through a cylinder (about one meter in length and .15 meter in diameter) filled with limestone chips to neutralize the sulfuric acid.

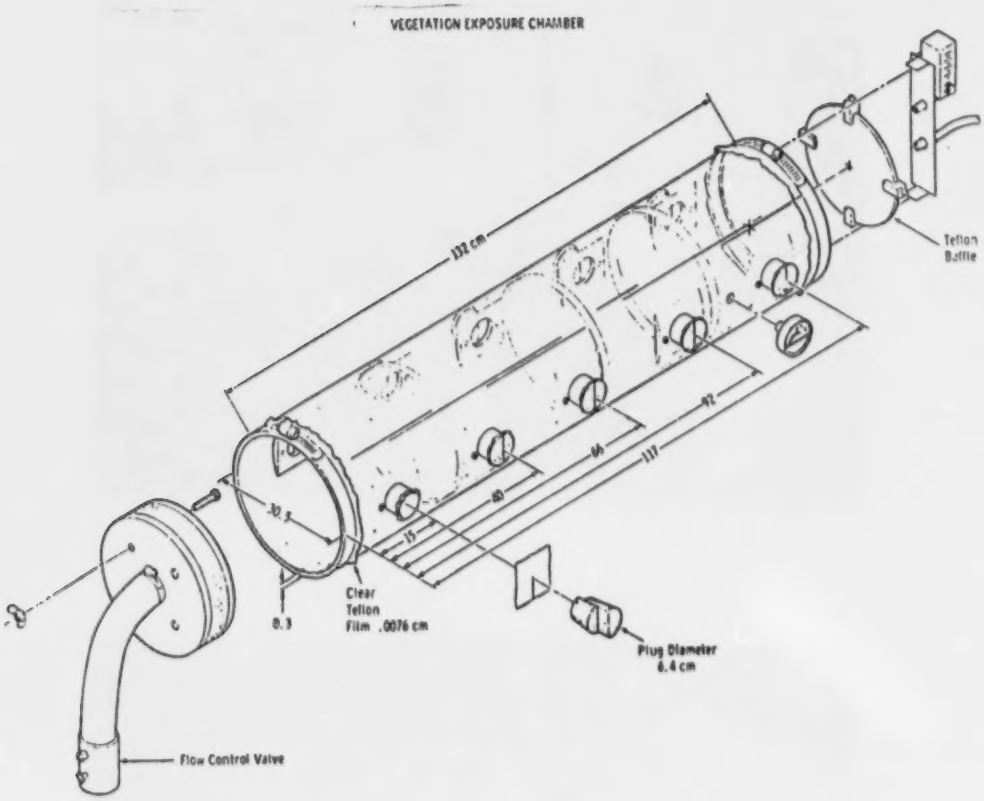
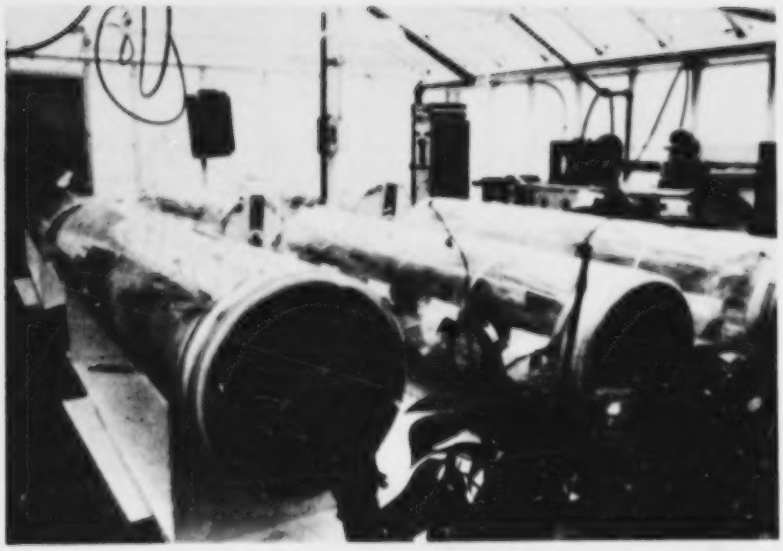
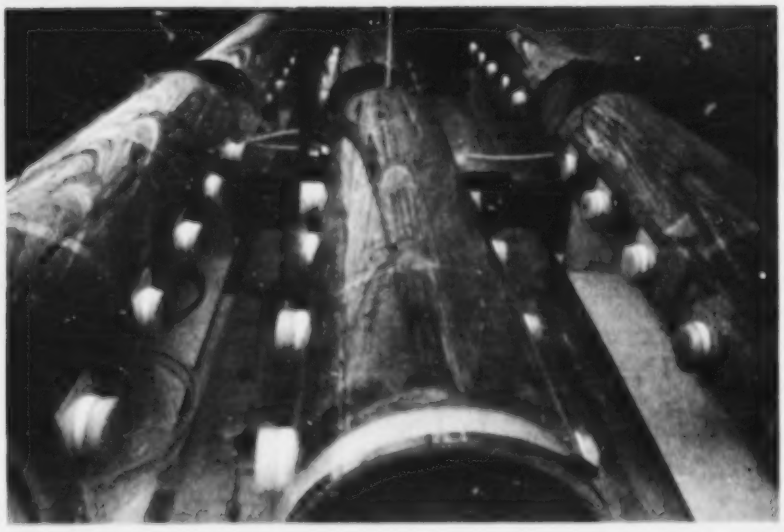


Figure 3. Construct on detail of single exposure chamber.





Figures 4A and 4B. Aerosol exposure chambers installed in greenhouse.

Mass flow in this system was balanced with a series of flow meters, valves and a variable transformer connected to an exhaust blower to control flow rates.

2.3 Flow Control

Initial exposures of vegetation to H_2SO_4 aerosol suggested the presence of static areas within the chambers. Varied aerosol concentrations were reflected by localized injury to vegetation in certain chamber positions. Baffles were developed to improve turbulent mixing within the chambers. The baffles were constructed of Teflon sheet material 3 mm thick. Square Teflon stock 1.2 cm was cut to 4 cm lengths and attached to circular sheet pieces 23 cm in diameter at 90 degree intervals. Holes were drilled in the ends of the square support brackets and fitted with a stainless steel spring which was compressed with a Teflon piston made from 4 mm diameter rod stock. The baffle assembly was then positioned in the chamber approximately 15 cm from the aerosol inlet. This design facilitated baffle removal during chamber cleaning and introduction of plants into the chamber. The baffles improved mixing, as indexed by reduced variation in symptoms. The relationship between mass flow within the exposure system and pressure drop monitored in the chambers was also improved with baffles.

Figures 5 and 6 show the relationship between velocity, pressure drop and mass flow in chambers with and without baffles. Each point represents the average of five velocity measurements at each location. The similar curves shown in Figure 6 suggest a more accurate prediction of mass flow than those of Figure 5. These curves were fitted to 4th order equations to provide exact fits of the observed data points.

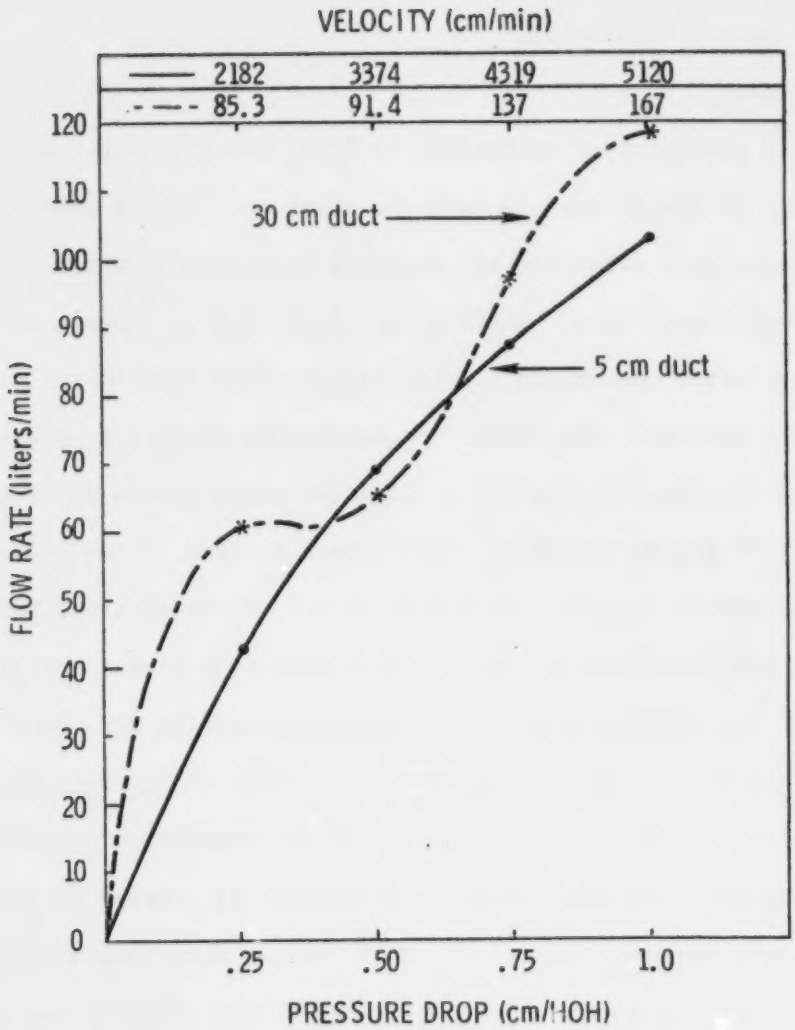


Figure 5. Mass flow measured through chamber without Teflon baffle.

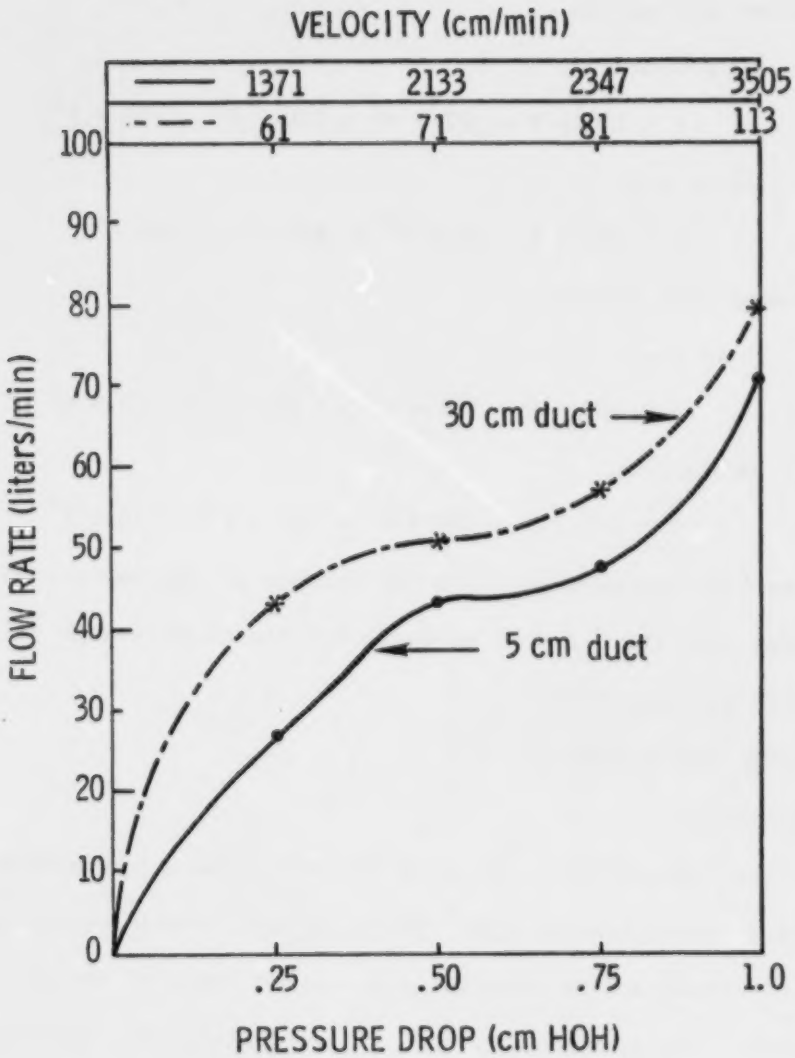


Figure 6. Mass flow measured through chamber with Teflon baffle installed.

The equations are as follows:

Chamber w/o baffle

5 cm duct

$$F = 250.6\ P - 377.7\ P^2 + 370.7\ P^3 - 139.7\ P^4$$

30 cm duct

$$F = 587.4\ P - 2010\ P^2 + 2851\ P^3 - 1300\ P^4$$

Chamber with baffle

5 cm duct

$$F = 107.9\ P + 108.4\ P^2 - 458.7\ P^3 + 313.6\ P^4$$

30 cm duct

$$F = 311.5\ P - 720.9\ P^2 + 705.6\ P^3 - 216.5\ P^4$$

Mass flow was calculated as the average of the two values determined from the 5 and 30 cm ducts for the chamber with baffle at a given pressure drop.

3. AEROSOL GENERATION

3.1 Equipment

Several methods were investigated for producing submicron aerosols of concentrated sulfuric acid. These included stabilized volatilization of liquid sulfur trioxide and various types of aerosol generators. The stabilized liquid SO_3 volatilization, although widely used, was not chosen for this project for three reasons. These were:

1) High output of H_2SO_4 aerosol was not required in our system
2) Liquid SO_3 is a hazardous material requiring elaborate safety precautions
3) The costs incurred in meeting objective 2 could not be satisfied by our current funding

A specialized aerosol generator was therefore selected which met our criteria for producing a substantial amount of particulate mass in the submicron range while providing sufficient aerosol concentration.

The aerosol generator we used is commercially available (ERC model 7330) and utilizes a three-stage system to produce the submicron aerosol. A collison type nebulizer (19) produces a fine aerosol by directing an atomization jet against a baffle, the finer aerosol particles are carried by upward air flow to an inertial impaction stage which removes particles larger than one micron, submicron particles are then carried through a Krypton 85 charge neutralization unit and into the exposure chamber. The generator is driven by compressed dry air at 3.2 kg/cm^2. Inertial size separation and charge neutralization minimize problems of condensation and liquid retention in the aerosol space beyond the generator (7).

3.2 Modifications

Problems have been encountered with this system. Corrosion of stainless steel components prior to the impaction stage of the generator occurs if the system is not cleaned immediately after use. This is due probably to the reaction of the acid aerosol with ambient humidity resulting in a more dilute but more reactive sulfuric acid film on the stainless steel surfaces. Careful monitoring of the system pressure and the subsequent flow rates is required to assure experimental reproducibility.

After a period of use it was determined necessary to replace the stainless steel collison nebulizer portion of the generator. Critical orifices in the stainless steel nebulizer were susceptible

to corrosion by sulfuric acid. Aerosol distributions were shifted to larger size particles. This was probably due to decreased jet velocities through the larger corroded orifices and subsequent decrease in impaction efficiency on the nebulizer baffle.

A Teflon nebulizer was built to specifications similar to those of the stainless steel unit. The Teflon nebulizer performed well apart from construction problems associated with machining Teflon to close tolerances. No significant change in aerosol size distribution was observed after prolonged use of the Teflon nebulizer. This modification is necessary for use of this type of generator in long term and/or intermittent H_2SO_4 aerosol production.

Most corrosion problems were associated with the nebulizer and impaction stages of this generator. Fine particles passing through the impactor produced very little corrosion. The neutralization stage also mitigated corrosive affects by decreasing surface deposition and particle agglomeration (15). There was no visible corrosion of stainless steel components beyond the aerosol generator.

4. AEROSOL CHARACTERIZATION

4.1 Inertial Impaction

Several methods were used to define the character of the exposure chamber aerosol. The principal method employed a seven stage rectangular jet impactor operated at 20 liters per minute. Glass fiber impaction surfaces were weighed immediately prior to sampling, the impactor was assembled and placed in an insulated box before and after use. Impaction discs were then reweighed and the percentage mass collected per stage was plotted versus equivalent aerodynamic

diameter on log-probability paper. Figure 7 shows typical aerosol size distributions for the two aerosol chambers. Note that these aerosol distributions are similar with mass mean diameters of 0.64 μm - 0.56 μm and standard geometric deviations of 1.72 - 1.70. These values are comparable to ambient sulfate aerosols. Figure 8 shows a typical particle volume distribution, the shaded area was estimated based on actual values shown by the vertical bars. When SO_3 is introduced into the atmosphere and undergoes reaction with ambient water vapor the H_2SO_4 particles formed grow and transition from the nuclei to accumulation size modes occurs (5,32). It is reasonable to assume that particles in the accumulation mode (0.15 to .5 μm diameter) will have the principal long range effect on vegetation because of their residence time in the atmosphere. This is especially pertinent when effects on remote environments are considered.

4.2 Electrical Aerosol Analyzer

A significant amount (about 25 percent) of the aerosol mass produced by the aerosol generator was found to be below conventional inertial impaction limits. Further characterization, therefore, required the application of another device to assay these particles (14). An electrical aerosol analyzer (EAA) was used to classify fine particles from 0.02 to 1 μm in diameter (16). However, a problem was encountered in using the EAA unit in the direct measurement of chamber aerosol. At concentrations above about 5 mg/m^3 the electrometer filter of the EAA became saturated and inoperative.

Concentrations greater than 5 mg/m^3 were needed to induce acute injury to vegetation. This problem necessitated the dilution of

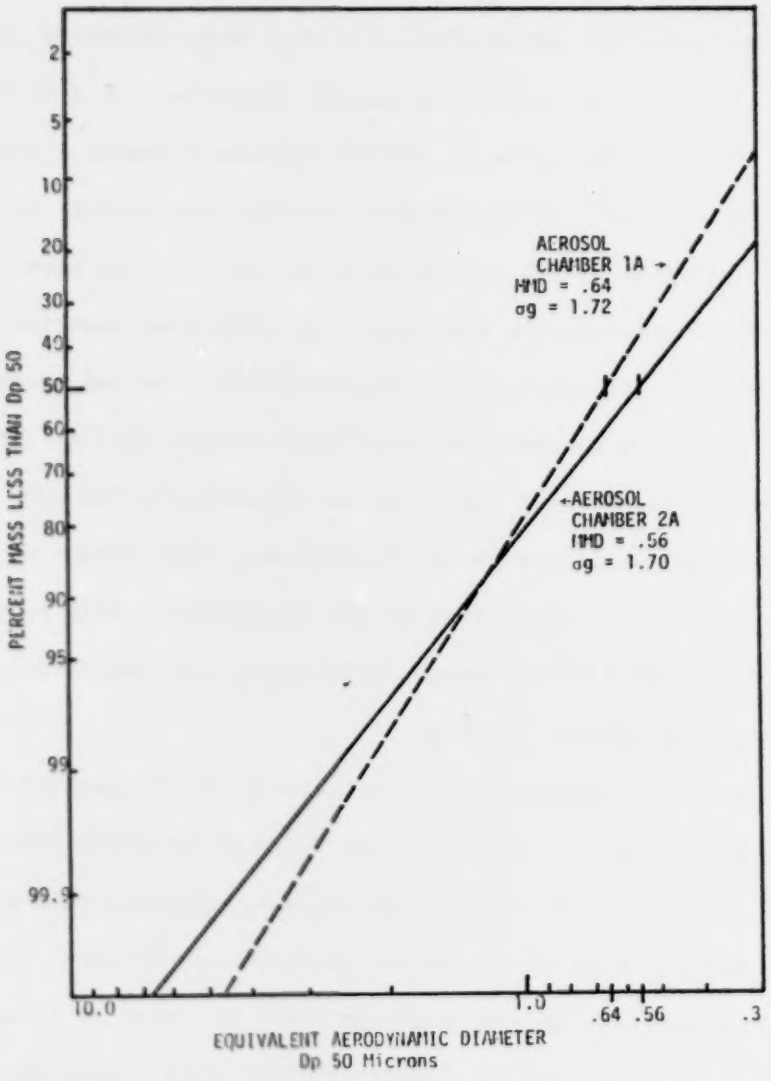


Figure 7. Typical aerosol size distribution in exposure chambers.

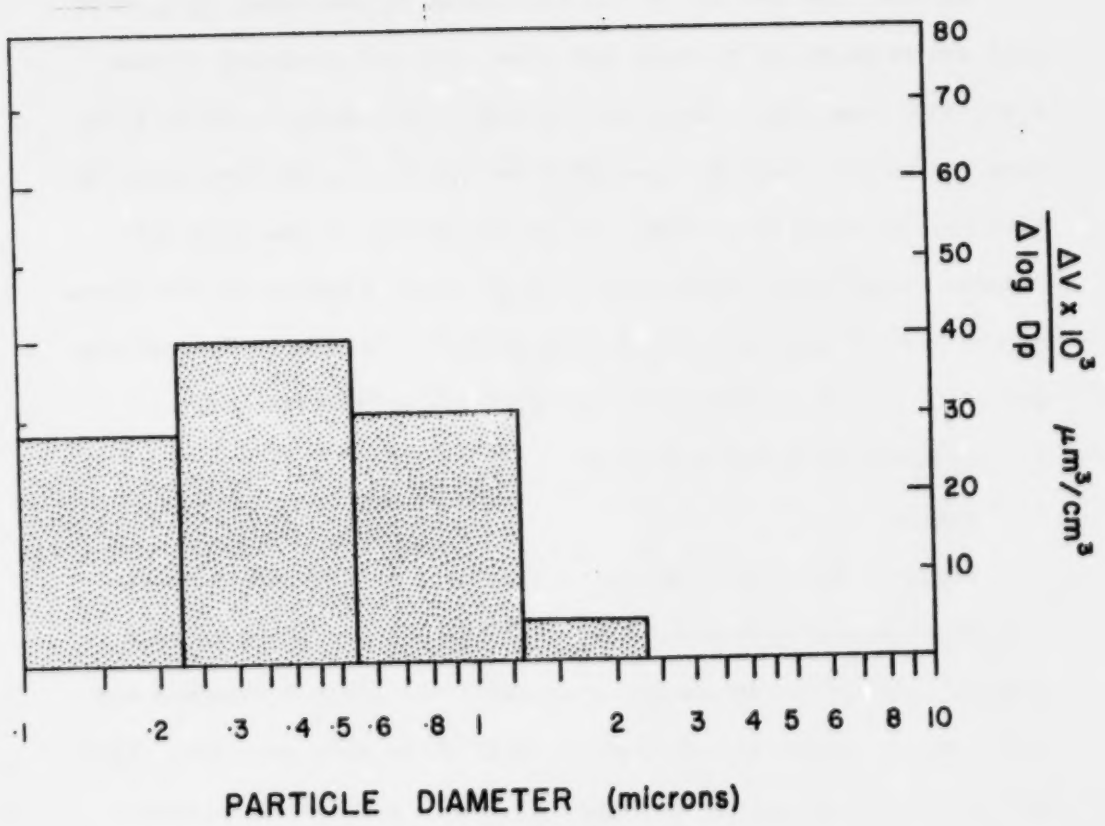


Figure 8. Aerosol volume distribution measured within exposure chambers.

chamber aerosol prior to EAA characterization. Dilution introduced a potentially significant variable. This problem is not expected to occur during chronic exposures at concentrations of less than 100 $\mu g/m^{-1}$ which could be monitored continuously and directed with the EAA.

4.3 Mass Flow Calculations

Aerosol mass per volume was calculated by measuring H_2SO_4 mass used and relating it to total air flow. It was necessary to make H_2SO_4 mass measurement using gravinimetric procedures. Total H_2SO_4 mass per volume could be calculated on this basis and then compared with that determined by summation of the masses on the impactor stages. Significant quantities of H_2SO_4 were retained as thin films on the aerosol generator components prior to the charge neutralizer and could not be reproducibly recovered for volumetric assay.

5. OPERATION OF EXPOSURE SYSTEM

5.1 Design

Figure 9 shows the logistic arrangement of components in the vegetation exposure system. Only two chambers are shown in this diagram. In the actual system there were two aerosol chambers and four control chambers connected in parallel in each position. This may be visualized better in Figure 1. There are five processes shown in Figure 9. These relate to aerosol generation, flow amendment, flow control, flow measurement and aerosol removal.

Details of our aerosol generation methods have been discussed in the previous section. Concentrated reagent grade sulfuric acid was weighed and placed in the nebulizer. Compressed dry air at 3.2 kg/cm^2 was used to drive the generator at 2.5 kg/cm^2. The aerosol was

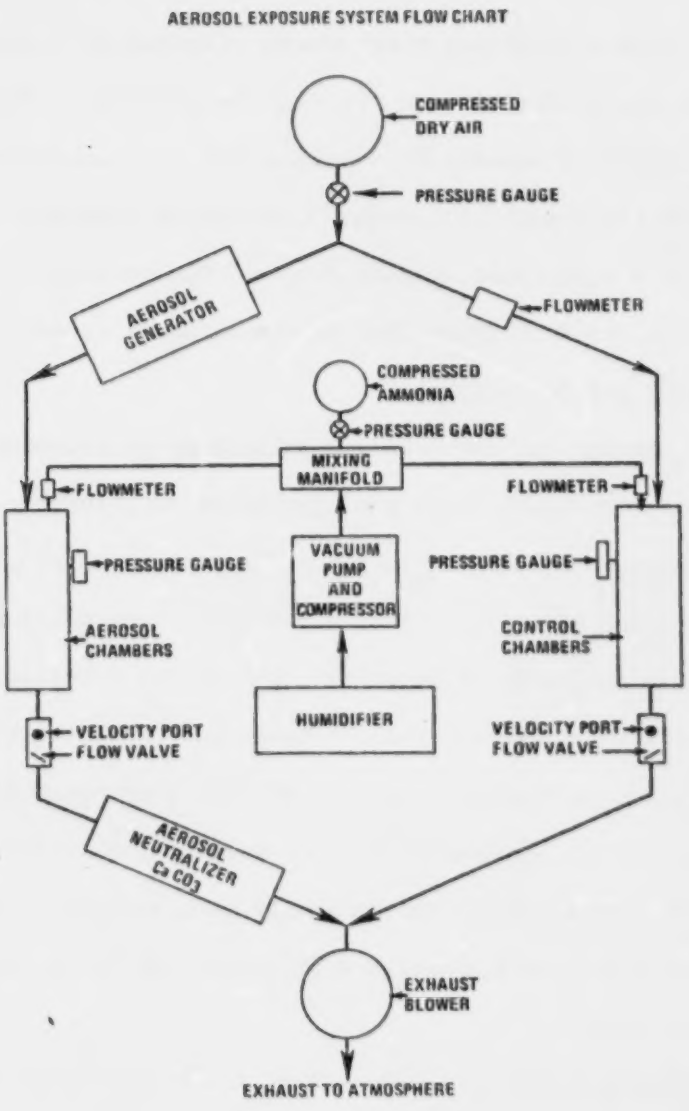


Figure 9. Flow chart showing logistic arrangement of components in system for exposing vegetation to sulfuric acid aerosol.

carried to the chambers through 1.25 cm Teflon tubing.

Chamber aerosol was amended with either water vapor or ammonia via a separate delivery system. Water saturated moist air was evacuated from a stainless steel plenum attached to a commercial humidifier and delivered to a distribution manifold. Figure 10A shows the modified humidifier. Ammonia was also delivered to the same distribution manifold, from a combination regulator - flow meter attached to a compressed ammonia source. The distribution manifold was connected to individual flow meters attached to each chamber.

5.2 Control and Measurement

Flow through the chambers was maintained by introducing the aerosol and amendments under a slight positive pressure. Flexible exhaust hoses were attached to the chambers by a short piece of stainless steel pipe fitted with a butterfly type valve and a 64 cm port through which an air velocity probe may be inserted. An exhaust blower was attached to the exhaust hoses and regulated with a variable power supply. Each chamber was fitted with a pressure drop gauge. Individual chamber pressures were balanced by the variable speed control and the butterfly valves in the exhaust lines. As discussed in section 2.3 chamber pressure drop could then be correlated with total mass flow.

Chamber aerosols were measured by inserting either the impactor (Figure 10B) or the sampling tube (EAA) through ports fitted to the chambers, as discussed in section 2.2. Problems with simultaneous characterization using inertial impaction and EAA were discussed previously. Flow in the chambers was measured by inserting a hot wire

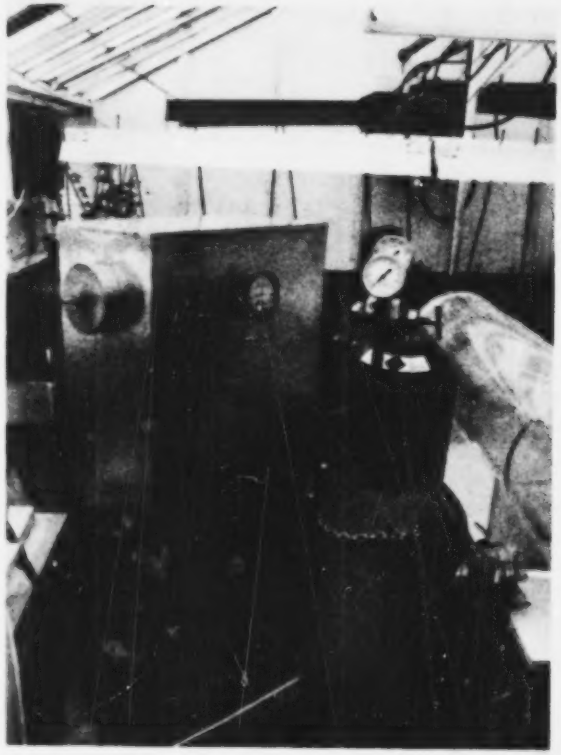


Figure 10A. Humidifier equipped with stainless steel plenum to provide moist air for maintaining chamber humidity.

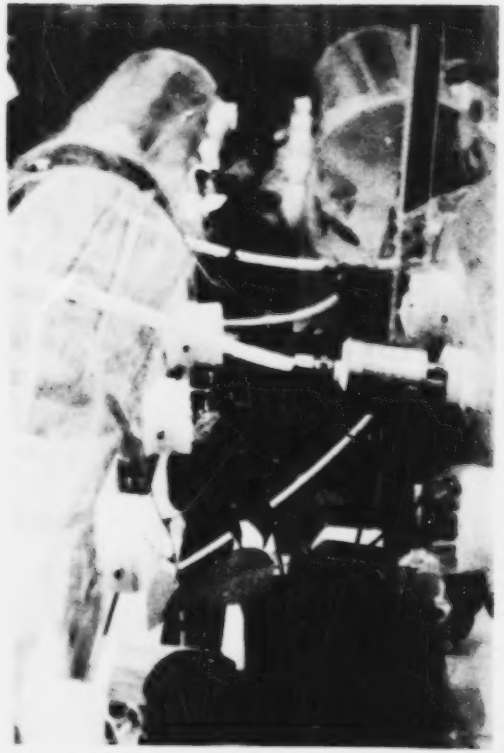


Figure 10B. Inertial impactor inserted through port in chamber for aerosol sampling.

probe through ports in the chamber and through the exhaust control valve and integrating the values obtained. Humidity within the system was measured using an aspirated wet and dry bulb psychrometer. Room humidity was monitored with a conventional hydrothermograph. A thermocouple psychrometer was tested for use in this system but probe design and calibration have not yet been perfected. The psychrometer has twelve individual channels and has the advantage of providing a near continuous record of humidity with the system. The principal disadvantages are the limited humidity range where operation is possible and complications caused by air flow around the sensors.

5.3 Aerosol Removal

Aerosols were removed from the exposure chambers by exhausting them through a stainless steel cylinder 100 cm by 15 cm filled with limestone chips. This unit effectively removed aerosols and did not introduce a large pressure drop in the aerosol exhaust line. Control and aerosol exhaust lines were connected together after the limestone filter and the filtered air was exhausted outside the greenhouse.

SECTION II

EFFECTS OF SUBMICRON SULFURIC ACID AEROSOLS ON VEGETATION

6. MATERIALS AND METHODS

The principal materials and methods relating to this research were covered in the preceding part of this thesis. These concerned generation, distribution and characterization of the sulfuric acid aerosols. Only those aspects relating to plant growth and study will be considered here.

6.1 Growth and Maintenance of Plants

Plants were grown in steamed or unsteamed soil mixtures consisting of two parts soil and one part sand volume per volume. Most plants were grown from seed in 10 cm square plastic pots with five seeds per pot. After emergence plants were thinned to three plants per pot removing nonuniform individuals. New foliage production was forced on woody species by removal of old foliage, root pruning and transplanting. Some woody species were regenerated by rooting stem cuttings. When this was not possible plants were exposed only once or as often as new foliage naturally developed.

Natural greenhouse lighting was supplemented with conventional and ultraviolet-enhanced fluorescent lamps. Photoperiods were maintained at 16 hours during the fall and winter. Plants were placed in chambers and covered with black plastic to evaluate the

effect of dark conditioning prior to exposure. During the summer, the entire greenhouse was shaded by applying a 50% spray coating of white shading compound to minimize solar heat loading.

6.2 Growth Measurement

Changes in plant growth after exposure were measured by carefully placing individual leaves in a specially designed glass frame upon which they could be traced. The same leaves were again traced after varied growth periods. The respective changes in growth were then quantified by measuring the area of the tracings using an area integrating digitizer. This procedure is non-destructive and allows the development of a continual growth record of individual leaves.

6.3 Sulfur Analysis

Foliar sulfur analyses were done by the Research Analytical Laboratory at the University of Minnesota. Bulk foliage samples, 0.5 to 5 g fresh weight basis, were collected from paired areas of individual chambers. Samples were air dried, ground and analyzed using a nitric/perchloric acid digestion - barium chloride precipitation procedure. Reproducibility is in the range of 10 to 15 percent for this method. Results are reported as parts per million total sulfur.

6.4 Scanning Electron Microscopy

Foliar samples were also examined with a scanning electron microscope. Leaf tissue was excised from healthy areas of control plants and asymptomatic and necrotic areas of exposed plants. Specimens were fixed in 5 percent glutaraldehyde and stored at 4°C for 72 hours. This was followed by 2-hour sequential dehydrations in 25, 50, 75 and 100 percent ethanol. Tissue was then dried using a

critical point drier, sectioned and mounted in a silver mounting compound. Mounts were then separated and those not intended for X-ray analysis were gold coated. X-ray emission spectra were obtained for those elements with greater atomic mass than sodium.

6.5 Injury Evaluation

Plants were rated according to the following scale, corresponding to visible injury induced by sulfuric acid aerosol:

0 - no visible injury

1 - less than 1 percent necrotic leaf tissue

2 - less than 10 percent necrotic leaf tissue

3 - greater than 25 percent necrotic leaf tissue

Injury was determined from 24 to 48 hours after the exposure, when the necrotic areas were clearly defined. Ratings were assigned based on a qualitative evaluation of all exposed foliage.

7. EXPOSURE CONDITIONS RELATED TO PLANT RESPONSE

The principal objective of this research effort was to compare the effects of sulfuric acid aerosols on several diverse plant species under uniform exposure conditions. Additionally, temperature, relative humidity, age and pollutant concentration were examined as variables in relation to the response of specific species. Plant response was evaluated both qualitatively and quantitatively. The quantitative data is presented in the section dealing with sulfur accumulation. Qualitative observations regarding the influence of traditional factors affecting pollutant-plant interactions are discussed here. The objective of this section is to indicate that there are presently unknown factors involved in determining plant response to sulfuric acid aerosol and

that traditional conditioning factors are not principally involved. Detailed data regarding visible injury is not presented because it was found to be highly variable and may be misleading. The following comments are subjective evaluations based on at least 100 observations per factor. Rationale for varied response to sulfate is discussed in a later section.

7.1 Temperature

Plants were exposed at temperatures ranging from 15° to 26°C. Most exposures were conducted at 22 ± 2°C. Within this temperature range there was no significant effect on symptom expression. Injury to pinto bean ranged from less than 1 percent to greater than 25 percent necrosis of exposed foliage. Similar plant responses with other species were obtained over this temperature range with some mitigation of symptom expression observed at the higher temperatures.

7.2 Relative Humidity

Most exposures were made at humidities between 40 and 60 percent. Symptom expression was essentially uniform within this range. Extreme humidity changes altered responses. When conditions approached saturation and free water condensed as droplets on the plant surface, necrotic islands appeared at the point of droplet contact. Low humidities which induced plant stress, possibly in conjunction with an overall moisture deficit, reduced symptom development.

7.3 Age

Plant species examined did not appear to be significantly altered in sensitivity with increased age. Injury to pinto bean expressed as necrotic areas of exposed foliage ranged from less than 1 percent for 11 and 21 day old plants to greater than 25 percent for 12 day old

plants. Seedlings of several species ranging in age from 1 to 8 weeks showed similar genotypic differences without apparent age differences. However, pinto bean plants exposed at the time of flowering showed somewhat more extensive injury than was observed on younger plants. Primary leaves were generally more sensitive to injury than were the trifoliolate leaves.

7.4 Time

Degree of symptom development and duration of exposure were not well defined. Injury to pinto bean varied from less than 1 percent to greater than 25 percent necrosis of exposed foliage during 4 hour exposures. Injury appeared to reach a maximum for a given species after a threshold time had been exceeded and then remained relatively unchanged for the duration of the exposure. This critical time varied between 2 and 8 hours. When injury occurred symptoms of water soaking were often evident during exposure and necrosis was apparent 16 to 24 hours after exposure.

7.5 Light

Light and dark conditioning prior to exposure was not extensively investigated. When plants were kept in the dark for 12 to 24 hours and then exposed, after one hour in light, symptoms were similar to a normal night-day regime. Plants were not exposed in complete darkness. The lack of response to light may be due to the controlling influence of a stable interstomatal CO_2 concentration which was not evaluated (22).

7.6 Concentration

Throughout this study an attempt was made to correlate macro-

symptom expression with the level of the factor inducing the response. We have found that concentrations of sulfuric acid aerosols below 10 mg/m^3 will not induce injury during short term (up to 12 hours) exposure in the species examined. Therefore, most exposures were made at concentrations of about one order of magnitude greater than this to characterize species sensitivity. A threshold response for most species apparently occurs between 10 and 100 mg/m^3 beyond which acute injury develops. Extremely high concentrations of 300 mg/m^3 induced necrosis which ranged from less than 1 percent to greater than 25 percent of exposed pinto bean foliage. Much additional work remains to be done to investigate chronic exposures which would be analogous to the ambient environment. These studies can only follow the characterization of subtle plant responses which can be substituted for visible symptoms.

7.7 Stress

Significant changes in plant metabolic and physiologic character are known to occur which are non-specific responses to stress (25). Responses which mitigated symptom expression were observed during the course of these studies. Specific studies to evaluate the effect of stressing plants prior to exposure were not done. However, it was noted that several factors could alter plant response if applied before exposure. High temperatures, insecticide application, attack by biotic pathogens, and moisture deficits appeared to reduce injury from sulfuric acid aerosol.

8. ACUTE PLANT RESPONSE TO SULFURIC ACID AEROSOL ON SELECTED SPECIES

8.1 Macrosymptoms

Previous reports of acid injury to vegetation have described localized necrosis which occurred at the point of droplet contact. Symptoms of this type were induced by large diameter droplets of acidic solutions deposited as rain or mist. The submicron acid aerosols used in this study are 2 to 3 orders of magnitude smaller than those previously reported and cause distinctly different symptoms.

Acute exposure to submicron sulfuric acid aerosols causes a marginal and tip necrosis of foliage which is similar to that caused by gaseous fluoride (see Figures 11A-13A). All plant species studied have shown a similar response which varies only in degree depending upon sensitivity. Extent of injury has varied between 0 and 50 percent necrosis of exposed foliage dependent upon exposure conditions and species of plant.

The onset of symptoms caused by acute exposure to sulfuric acid aerosol is relatively rapid. Water soaking is often apparent during four hour exposures to concentrations of 100 mg/m^3. Necrosis of leaf tips and margins is apparent after 12 to 16 hours and well defined after 24 to 36 hours. The following susceptibility classification is based on 8 hour exposures to sulfuric acid aerosol concentrations of 100 mg/m^3. Species are listed in order of decreasing sensitivity.

8.1-1 Bean--

Pinto bean (*Phaseolus vulgaris* L.) was the most studied and is one of the bean cultivars most sensitive to injury when exposed to

sulfuric acid aerosol. Bountiful bean was equally sensitive to this pollutant. Both of these plants developed more severe symptoms and showed injury earlier than other species exposed at the same time. Navy bean was also found sensitive but to a lesser degree than the other two bean cultivars. Injury to bean typically ranged from 5 to 25 percent of the exposed foliage depending on exposure conditions. Symptoms appeared first at the leaf edges and spread inward. The primary leaves of bean were somewhat more sensitive than were the trifoliolate leaves. However, symptoms were usually apparent on most of the exposed foliage with most rapidly expanding leaves showing the greatest degree of injury. Figures 11A and B show trifoliolate leaves of pinto bean with symptoms typical of sulfuric acid aerosol injury.

8.1-2 Poplar--

Hybrid poplar (Populus detoides Marsh. var.) was found more sensitive than the other deciduous species to sulfuric acid aerosol injury. The response of hybrid poplar varied from sensitive when first exposed at midseason to moderately resistant after a second flush of foliage was forced during midwinter. It is difficult to attribute this change in response to any particular factor at this time. There are several problems associated with the definition of reproducible experimental methods for perennial plants. Forced foliage production may also induce substantial physiological changes which could be confounded by seasonal alterations. This rationale makes the winter data questionable and we consider the sensitive classification of this species, based upon midsummer data, valid.

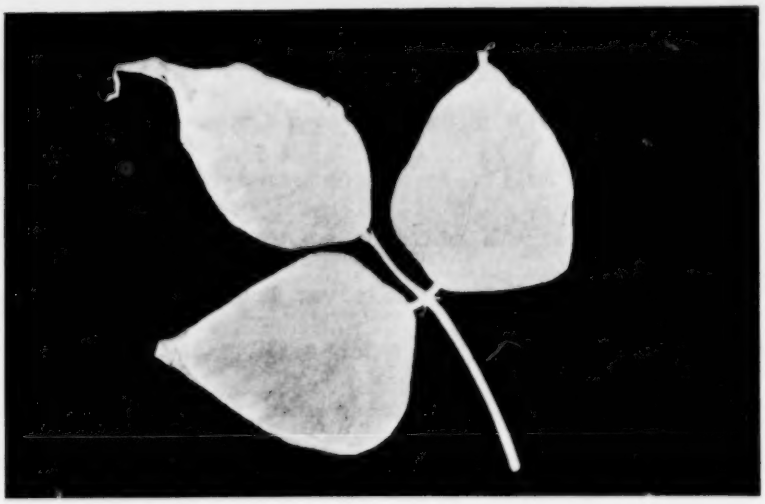


Figure 11A. Injury to trifoliolate leaf of pinto bean caused by sulfuric acid aerosol. Note: marginal and tip necrosis.

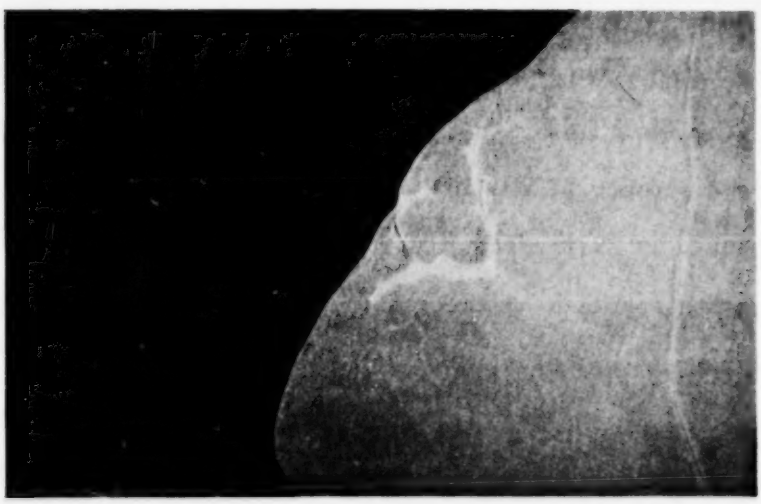


Figure 11B. Injury to primary leaf of pinto bean caused by sulfuric acid aerosol. Note: veinal necrosis near leaf margin.

Typical injury to hybrid poplar is shown in Figure 12B.

8.1-3 Soybean--

Soybean (Glycine max [L.] Merr.) was found to be moderately resistant to sulfuric acid aerosol injury. Some difference in response among soybean cultivars was observed but all were more resistant than was bean. In addition to necrosis of leaf tips and margins, exposed soybeans of the cultivar Hodgson developed a cupped leaf character somewhat resembling herbicide injury. Soybean seldom developed necrosis which affected more than 5 percent of the individual leaf area. Injury was usually restricted to a 1-2 mm margin at the leaf edge. Typical symptoms of sulfuric acid aerosol injury on soybean are shown in Figure 12A.

8.1-4 Ash and Birch--

Green ash (Fraxinus pennsylvanica Marsh.) and birch (Betula paperifera Marsh.) were more resistant than soybean to sulfuric acid aerosol. Injury was usually restricted to leaf tips and seldom exceeded one percent of the individual leaf area. Leaflets on ash showed relatively uniform tip necrosis without apparent difference due to age and position on the leaf. These species were exposed only once per season per plant. Forced foliage production could not be initiated as in the case of hybrid poplar. Different plants exposed during different seasons showed similar symptom development.

8.1-5 Corn and Wheat--

Corn (Zea mays L.) and wheat (Triticum aestivum L.) were the most resistant species exposed to the sulfuric acid aerosols. Both species showed some injury but it was usually restricted to a limited

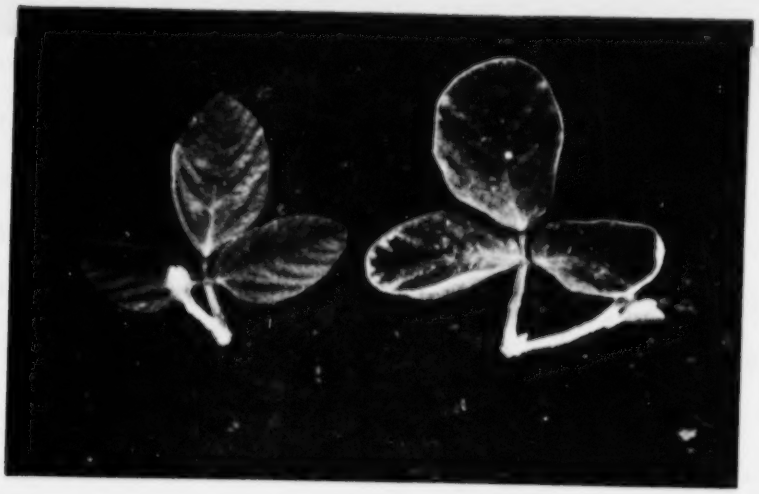


Figure 12A. Injury to soybean caused by exposure to sulfuric acid aerosol. Note: marginal necrosis of exposed leaf (right) compared to control (left).

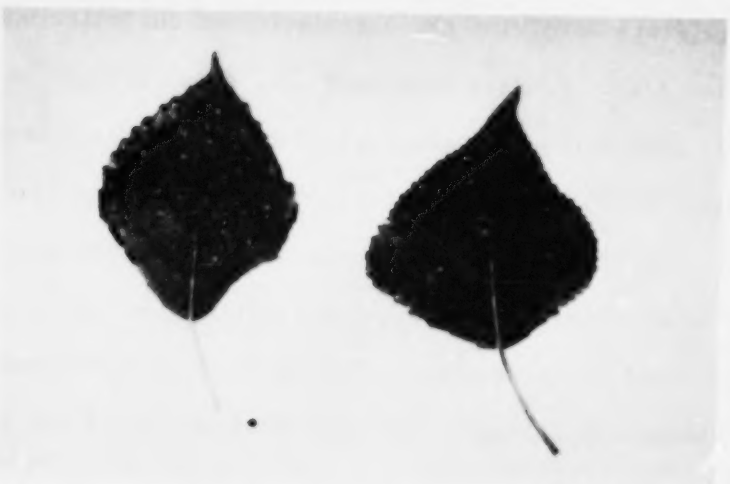


Figure 12B. Injury to hybrid poplar caused by exposure to sulfuric acid aerosol. Note: marginal and tip necrosis of exposed leaf (left) compared to control (right).

margin of 1 mm or less at the leaf edge. Exposed and nonexposed corn leaves are shown in Figure 13A. We do not yet know if this could be considered a general response of the Gramineae compared to broad leaf species. Both species appeared to grow normally after exposure and individual leaf necrosis did not exceed one percent of the individual leaf area. Corn and wheat were not examined for subtle responses to sulfuric acid aerosol injury. Initial plant exposures were made by inserting opposite plant branches through ports in the aerosol and control chambers as shown in Figure 13B. Subsequent studies with entire plants in the chamber gave similar results.

9. SUBTLE PLANT RESPONSES TO SULFURIC ACID AEROSOLS

9.1 Effect on Leaf Epidermis

Several recent reports on pollutant-plant interaction have described effects involving the degradation of cuticular waxes on the leaf surface (26). Studies were conducted to ascertain the effect of sulfuric acid aerosol on the cuticular layer. Pinto bean plants were exposed to 100 mg/m^3 of sulfuric acid aerosol and fixed for SEM study. The leaf cuticle was found to be generally unaffected except in areas where injury was macroscopically evident. Severe injury was found in these areas. Figure 14 shows untreated (A) and aerosol exposed (B) foliage. The epidermal cells and the guard cells collapsed on the exposed foliage. Figure 15 shows a normal stomate (A) and a stomate exposed to sulfuric acid aerosol (B). This degree of injury is permanent and renders the affected tissue nonfunctional. Although severe injury to the leaf is incurred, the cuticle wax does not appear to be undergoing degradation. The acute collapse

Figure 13A. Injury to corn caused by exposure to sulfuric acid aerosol. Note: exposed leaf (left) compared to control leaf (right).

Figure 13B. Opposite branches of plant inserted through ports in control chamber (left) and aerosol chamber (right).

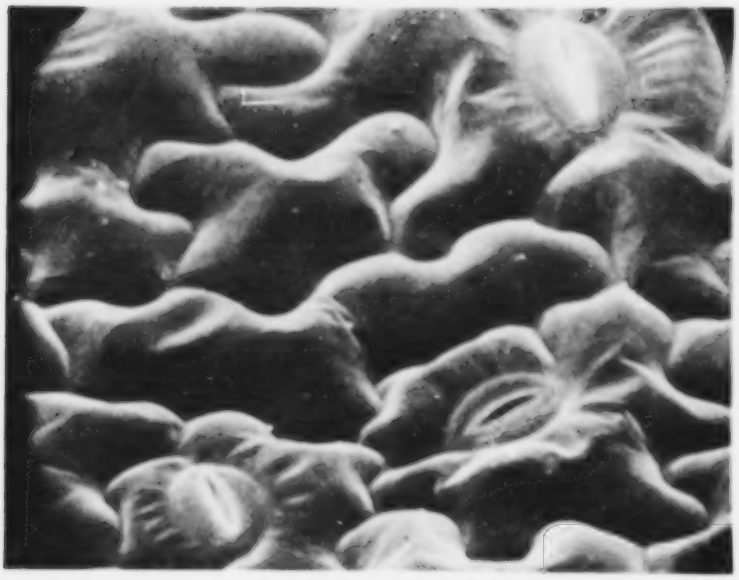


Figure 14A. Scanning electron micrograph of non-exposed leaf of pinto bean. Note: condition of stomates and cuticle.

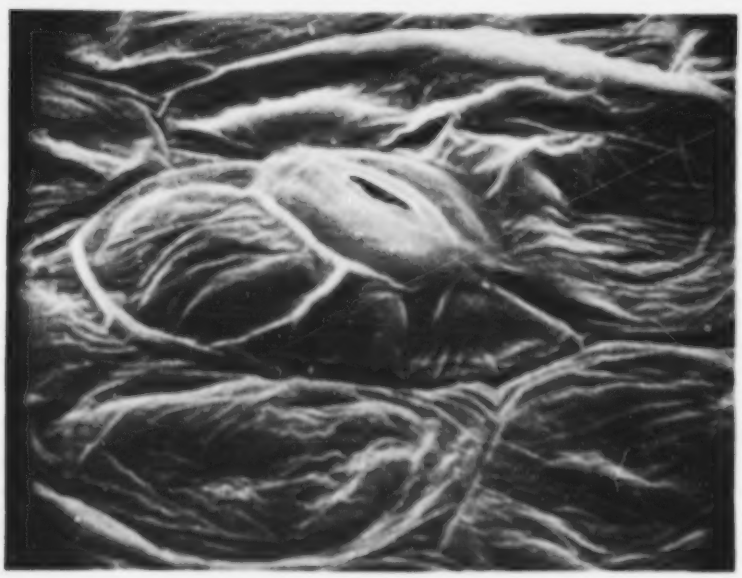


Figure 14B. Scanning electron micrograph of aerosol exposed leaf of pinto bean. Note: collapsed epidermal cells.

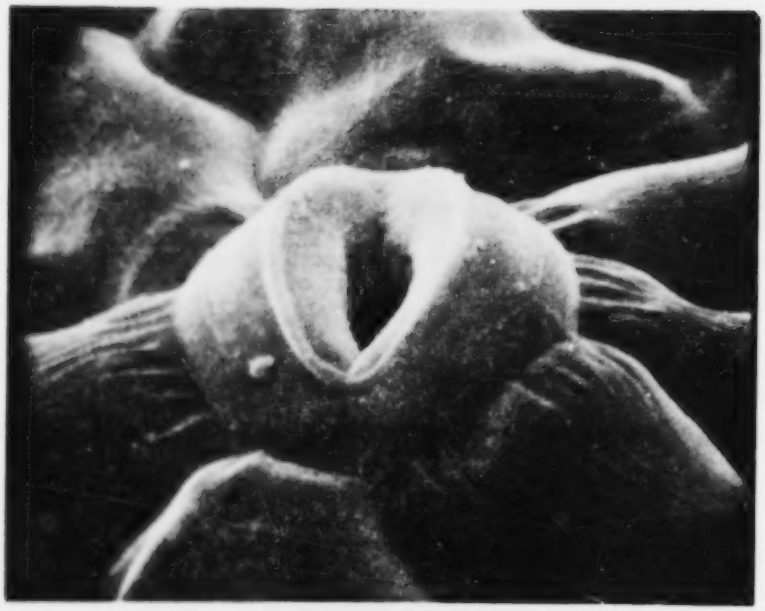


Figure 15 A. Scanning electron micrograph of healthy stomate of pinto bean.

Figure 15 B. Scanning electron micrograph of stomate exposed to sulfuric acid aerosol. Note: collapsed guard cells.

of epidermal cells can be better visualized in Figure 16 which shows normal (A) and exposed (B) leaves.

9.2 Elemental Composition

Figure 16C shows the elemental distribution of the surfaces shown in Figure 16A and B. The solid line represents the untreated and the dotted line the exposed foliage. The similar elemental composition indicates that sulfate is not being retained in the cuticle. Figure 17A shows an aerosol exposed leaf in cross section. The elemental composition of the surface (solid line) and interior (dotted line) indicate the areas which are shown in Figure 17B. Significantly more phosphorous and sulfur were found in the leaf interior. This may indicate that aerosol sulfate is being accumulated within the leaf.

9.3 Surface Particles

Efforts were made to assay the elemental composition of particles found on the leaf surface. Figure 18A shows a large (5 μm) particle which contains sulfur as a major constituent. Figure 18B shows the elemental composition of the particle shown in Figure 18A. Figure 19 shows the elemental composition of another particle, which is probably a silicate of aluminum, found near the particle in Figure 18A. Varied particle compositions such as these were commonly found. A predominance of sulfur particulates was not observed.

9.4 Changes in Foliar Sulfur Content

Several plant species were found to accumulate sulfur during 4 to 12 hour exposures to sulfuric acid aerosols ranging in concentration from 100 to 200 mg/m^3. Sulfur uptake by plants did not appear to be

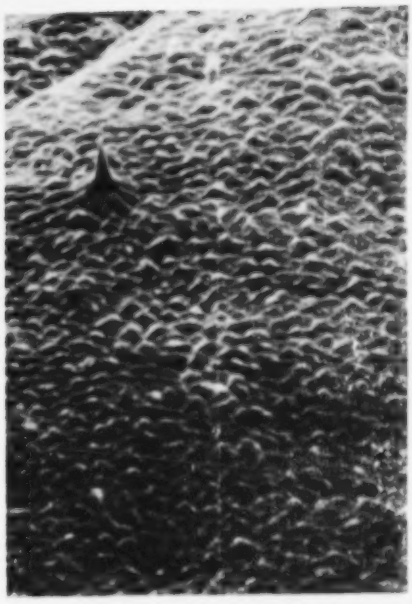


Figure 16A. Scanning electron micrograph of leaf surface of healthy pinto bean.

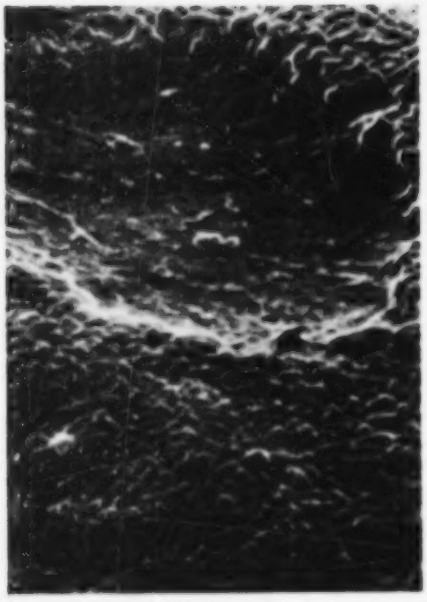


Figure 16B. Scanning electron micrograph of leaf surface of pinto bean exposed to sulfuric acid aerosol. Note: necrotic pit caused by droplet from sulfuric acid aerosol condensation.

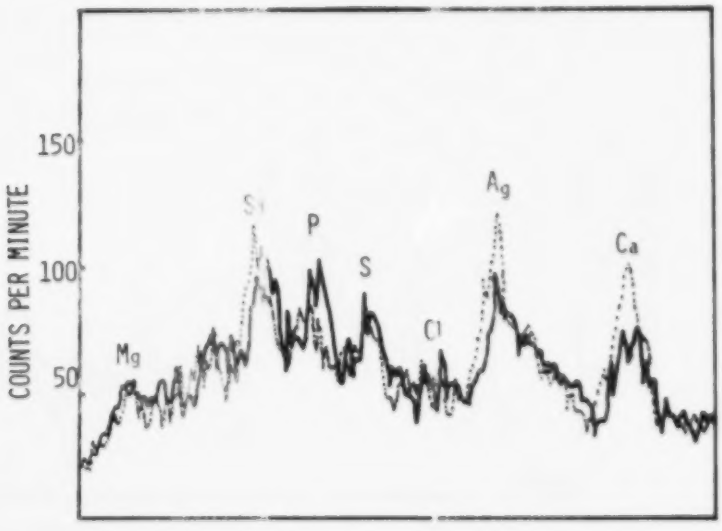


Figure 16C. X-ray microanalysis spectra of leaf surfaces of pinto bean. Note: similar elemental composition of surfaces exposed to sulfuric acid aerosol (dotted line) and control surface (solid line).

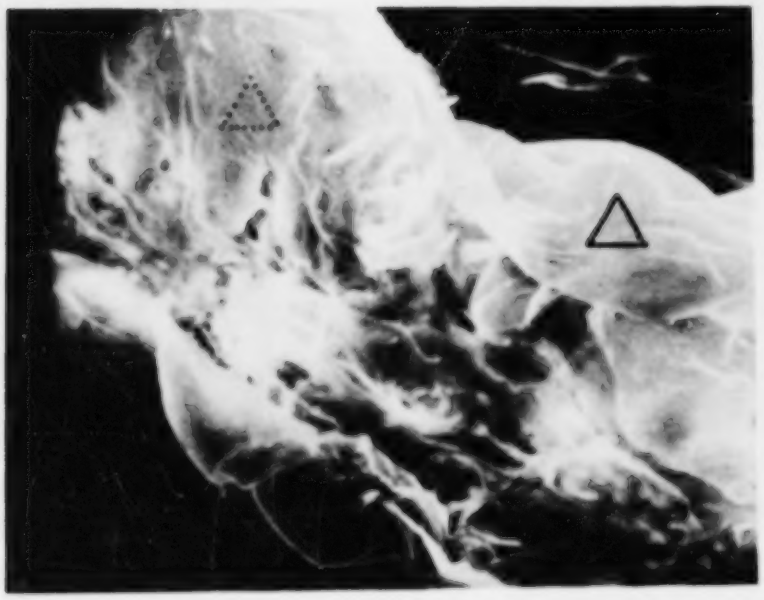


Figure 17A. Cross section of pinto bean leaf exposed to sulfuric acid aerosol. Note: epidermal surface (solid triangle) and interior surface (dotted triangle).

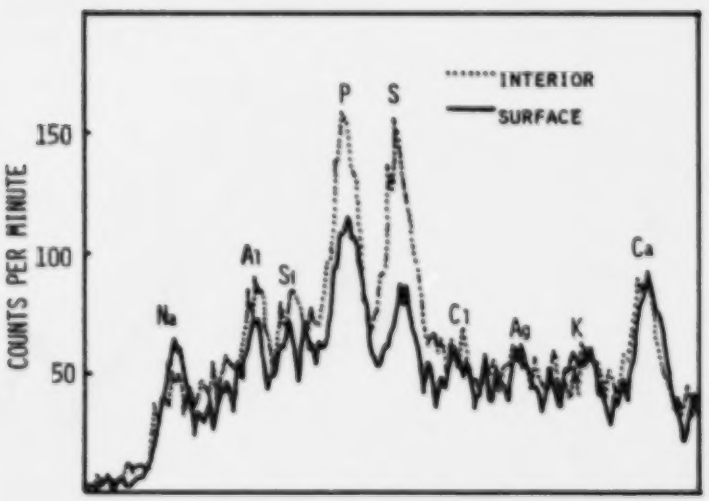


Figure 17B. X-ray microanalysis spectra of leaf shown in Figure 17A. Note: increased concentrations of sulfur and phosphorous found in leaf interior (dotted line) compared to exterior surface (solid line).

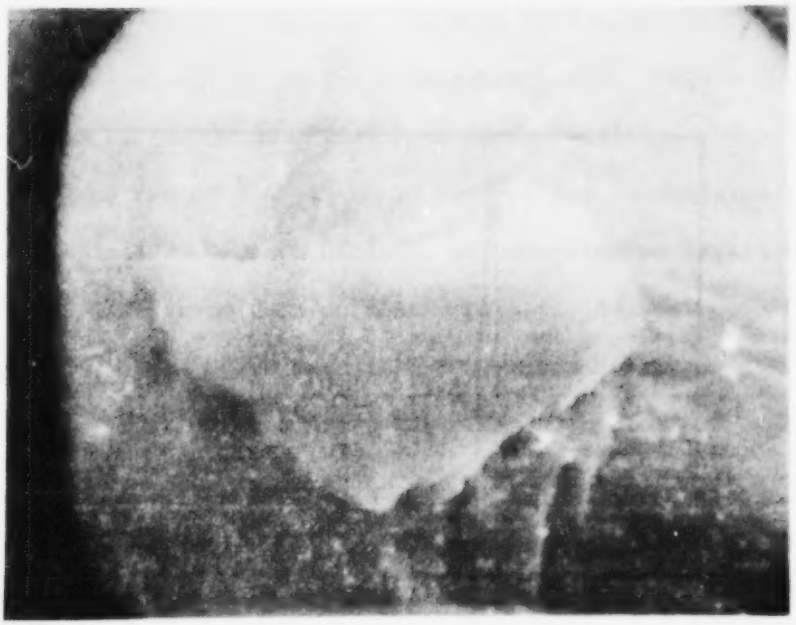


Figure 18A. Scanning electron micrograph of 5 μm particle found on surface of leaf exposed to sulfuric acid aerosol.

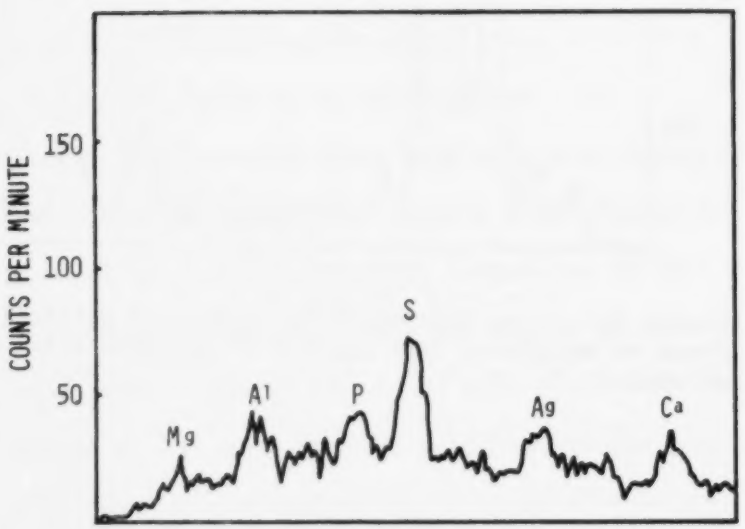


Figure 18B. X-ray microanalysis spectra of particle shown in Figure 18 A.

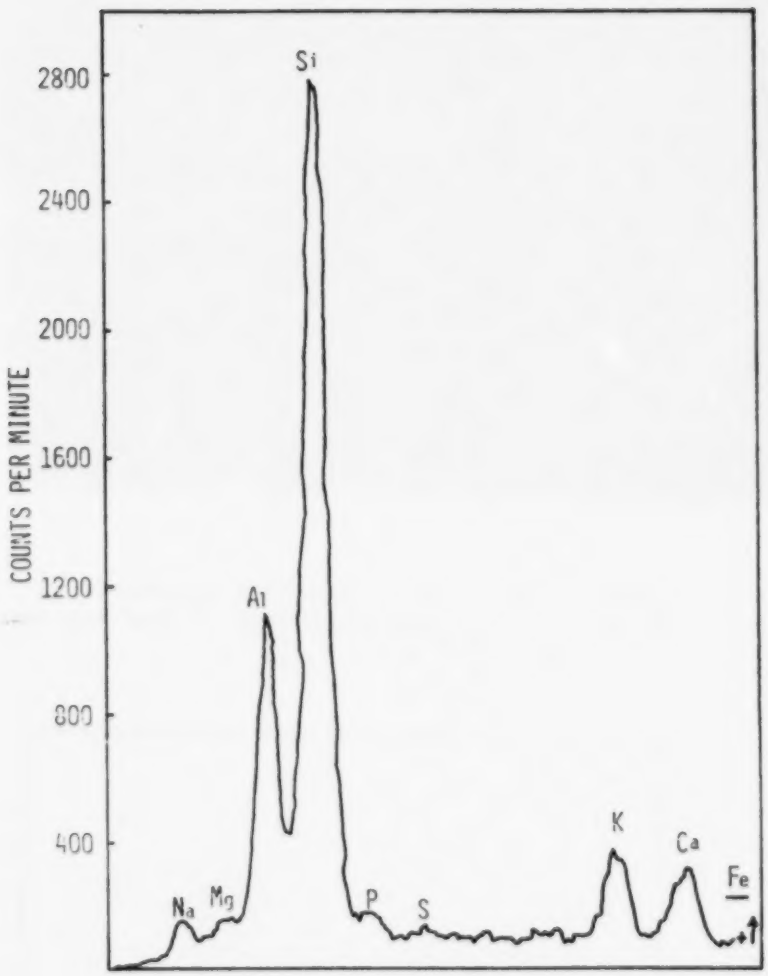


Figure 19. X-ray microanalysis spectra of particle found on surface of leaf exposed to sulfuric acid aerosol.

as much a function of aerosol concentration and duration of exposure as it did other unknown factors. Data on foliar sulfur content are shown for several plant species in Figures 20-24. Figure 20 shows sulfur accumulation in Bountiful bean on four different exposure dates. Plants ranged from 15 to 30 days of age depending on the specific exposure. On each occasion aerosol-treated plants had higher foliar sulfur contents than untreated plants. As shown on the 7-27-77 exposure this amounted to more than a twofold increase from 2000 to 4180 ppm. Most species grown from seed and exposed at about the same age showed an increase in background sulfur and a decrease in total sulfur accumulated during exposure as the season progressed. The reason for this is unknown. Figures 20-24 are comparable since the plant species in question were simultaneously exposed on the designated date. The season trend (showing decreased sulfur accumulation) is apparent for all species except hybrid poplar which showed an unusually high level of natural sulfur. Since exposure conditions were not exactly reproduced on the dates shown in Figures 20-24 other factors may be influencing the seasonal trend.

Additional studies using pinto bean were done midwinter, 1977-78 in an attempt to better define the factors affecting sulfur accumulation. Figure 24 shows pinto bean data comparable to that shown in Figures 20-23. Figures 25-27 show the effect of sequential sulfuric acid aerosol exposure on pinto bean at 48 hour intervals at a concentration of 250 mg/m^3 with 7, 9 and 11 day old plants, respectively. Data are presented for two control and two aerosol chambers with two subsamples for each chamber. Adjacent bars of the

same magnitude indicate insufficient foliage was available for subsampling. After the third exposure (Figure 27) the foliar sulfur content of exposed foliage has approximately doubled. A response of similar degree was achieved with one third this dose (concentration X time) (3) during midsummer (see Figure 24).

Pinto bean plants of different ages were exposed for 5 and 7 hour periods to about 175 mg/m^3 sulfuric acid aerosol. No apparent differences in sulfur accumulation were found. These data are presented in Figures 28-31 with the same subsampling format as previously described. Figure 28 and 29 show foliar sulfur contents for 16 and 26-day-old plants, respectively, exposed for 7 hours. The values shown are within the limits of analytical error. Figures 30 and 31 show the effect of 5-hour exposures on 19 and 43-day-old plants. These data are also similar within the limits of experimental error. A decreased response to increased aerosol concentration was also noted during the midwinter studies. Figures 32 and 33 show the effect of more than doubling the aerosol dosage on sulfur accumulation. Figure 32 shows the foliar sulfur content of pinto bean plants exposed to 175 mg/m^3 for 8 hours compared to control plants. Figure 33 shows the results of 12-hour exposure with an increased concentration of 390 mg/m^3. Differences in response are not apparent.

These results indicate that there are substantial seasonal differences in plant response to sulfuric acid aerosol stress. Plant age, and aerosol concentration did not markedly influence plant response during the midwinter studies yet similar plants accumulated large amounts of sulfur during midsummer. Lack of predictable response to

increased pollutant dosages may indicate that uptake of this pollutant is not following simple diffusion processes, and that presently unknown factors may be controlling plant response.

9.5 Change in Growth

Changes in individual leaf area following exposure to sulfuric acid aerosol were measured using a procedure described previously. Figure 34 shows the growth of exposed and non-exposed trifoliolate leaves of pinto bean as a function of initial leaf area. Young leaves exposed to 175 mg/m^3 for 7 hours showed a slight ($P = .25$) stimulation in growth which decreased as the initial leaf area increased beyond 75 cm^2. Growth of older trifoliolate leaves with similar initial areas was essentially unchanged after the aerosol treatment (Figure 35).

Paired primary leaves of pinto bean of similar initial area responded as did the paired trifoliolate leaves. Figure 36 shows the growth response of the primary leaves over a 2-week period following sequential exposures 1 week apart. Exposure to sulfuric acid aerosol did not show consistent effects on primary leaf development.

9.6 Plant Reproductivity

Pinto bean plants were exposed at time of flowering to 150 mg/m^3 of sulfuric acid aerosol for 5 hours on two consecutive days. Visual injury was apparent on foliage but not on flowers. Pollen viability was not assessed and it was not possible to make an accurate assessment of effect on yield. Since macroscopic effects on flowers were not observed and investigation of subtle effects on reproductivity require longer term studies, more research needs to be done to define this effect.

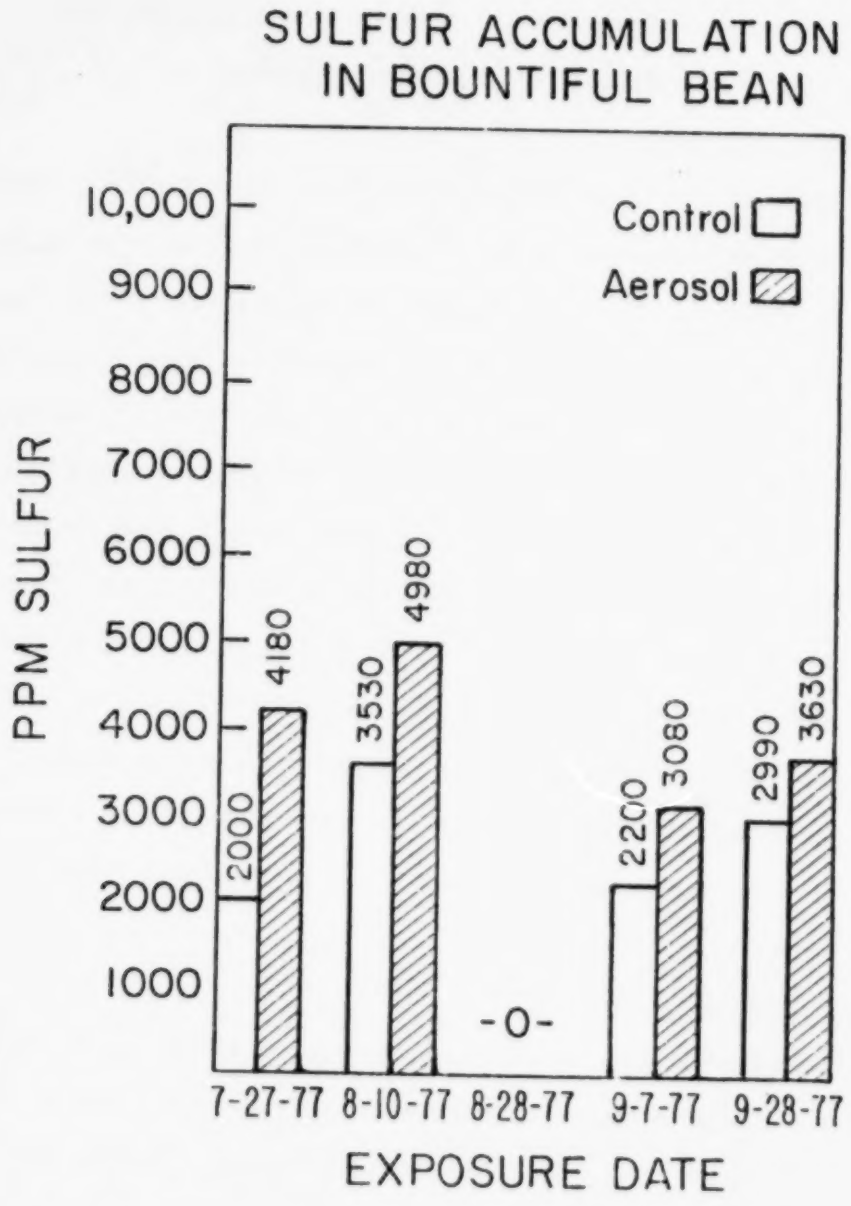


Figure 20. Foliar sulfur accumulation in Bountiful bean.
Note: seasonal trend showing decreased sulfur accumulation.

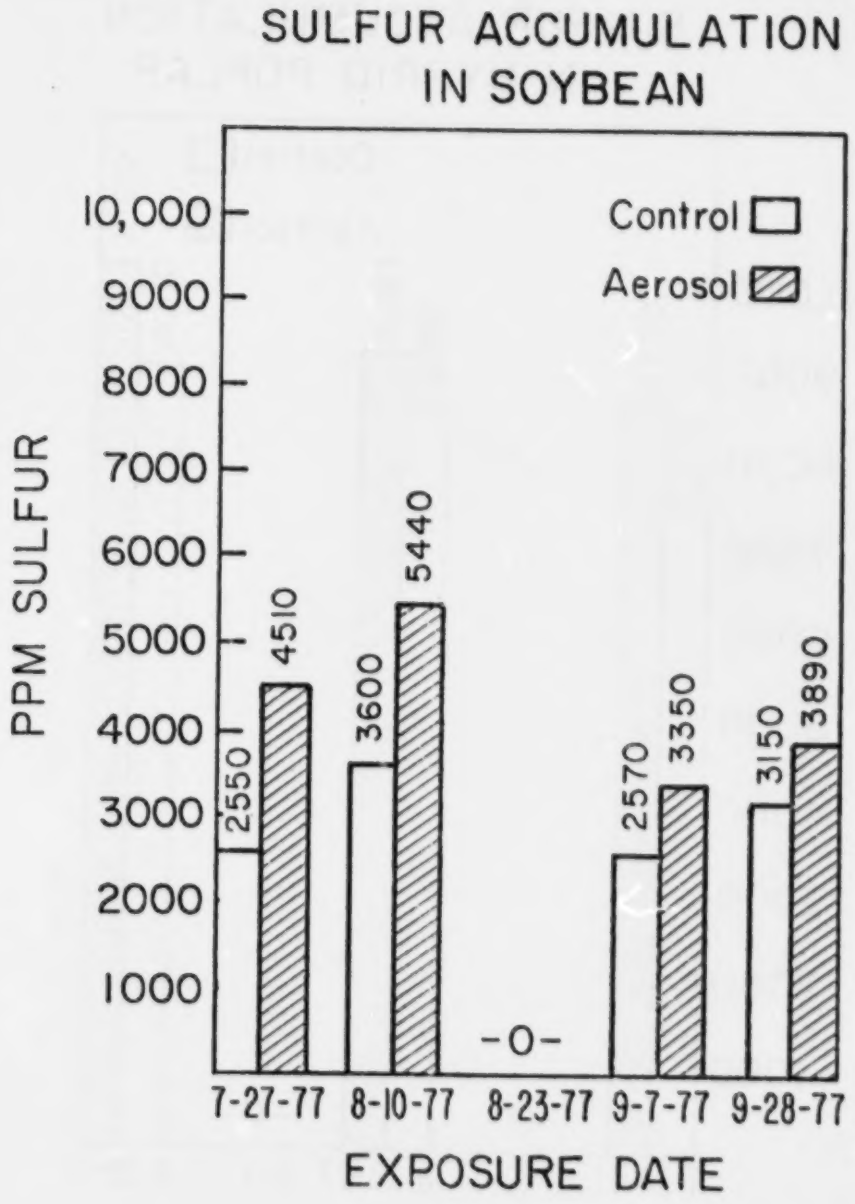


Figure 21. Foliar sulfur accumulation in soybean.
Note: seasonal trend showing decreased sulfur accumulation.

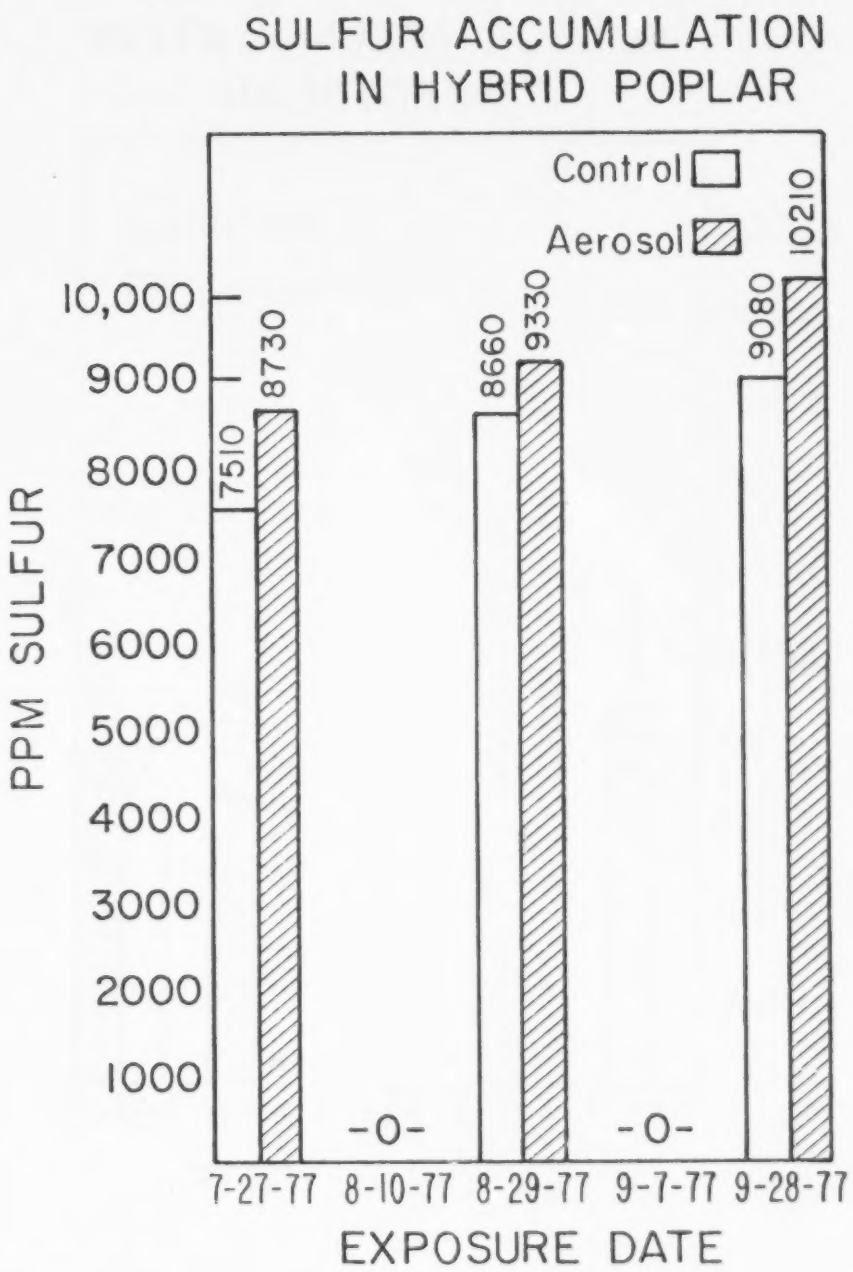


Figure 22. Foliar sulfur accumulation in hybrid poplar.

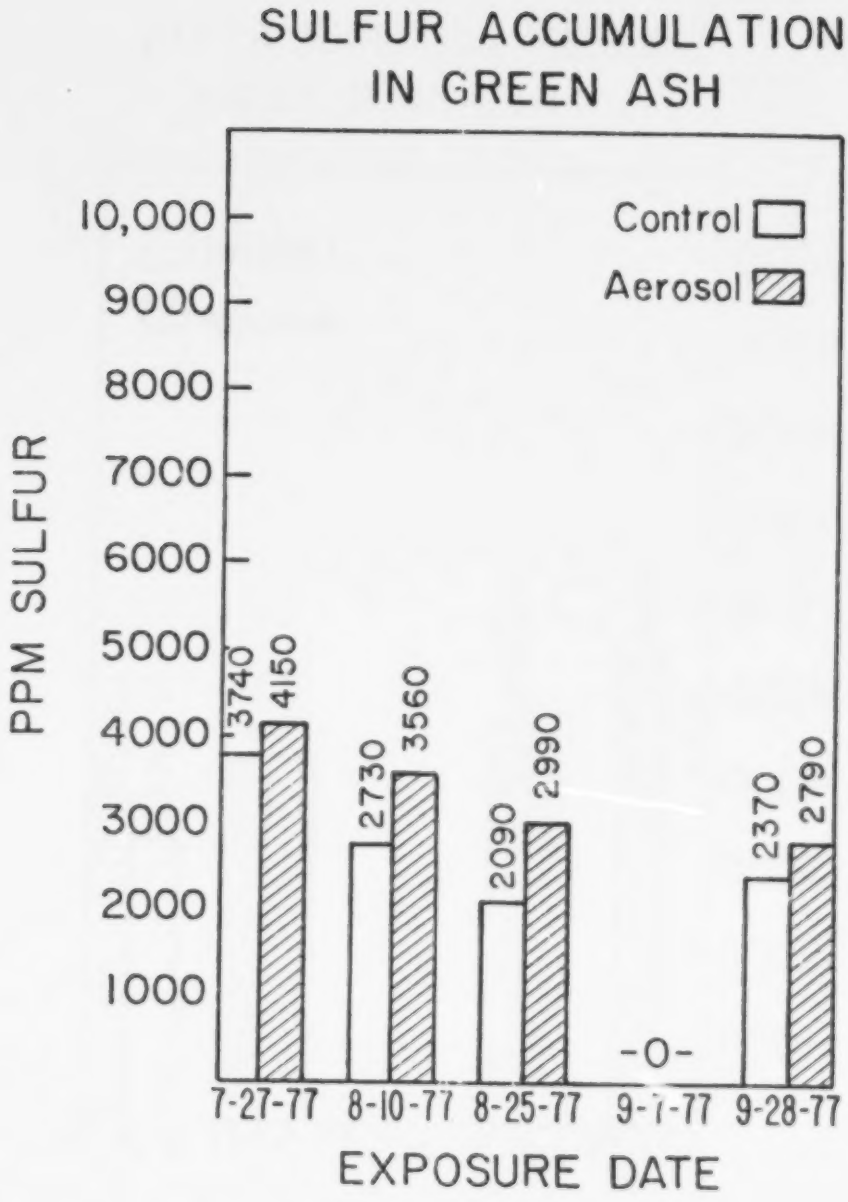


Figure 23. Foliar sulfur accumulation in green ash.

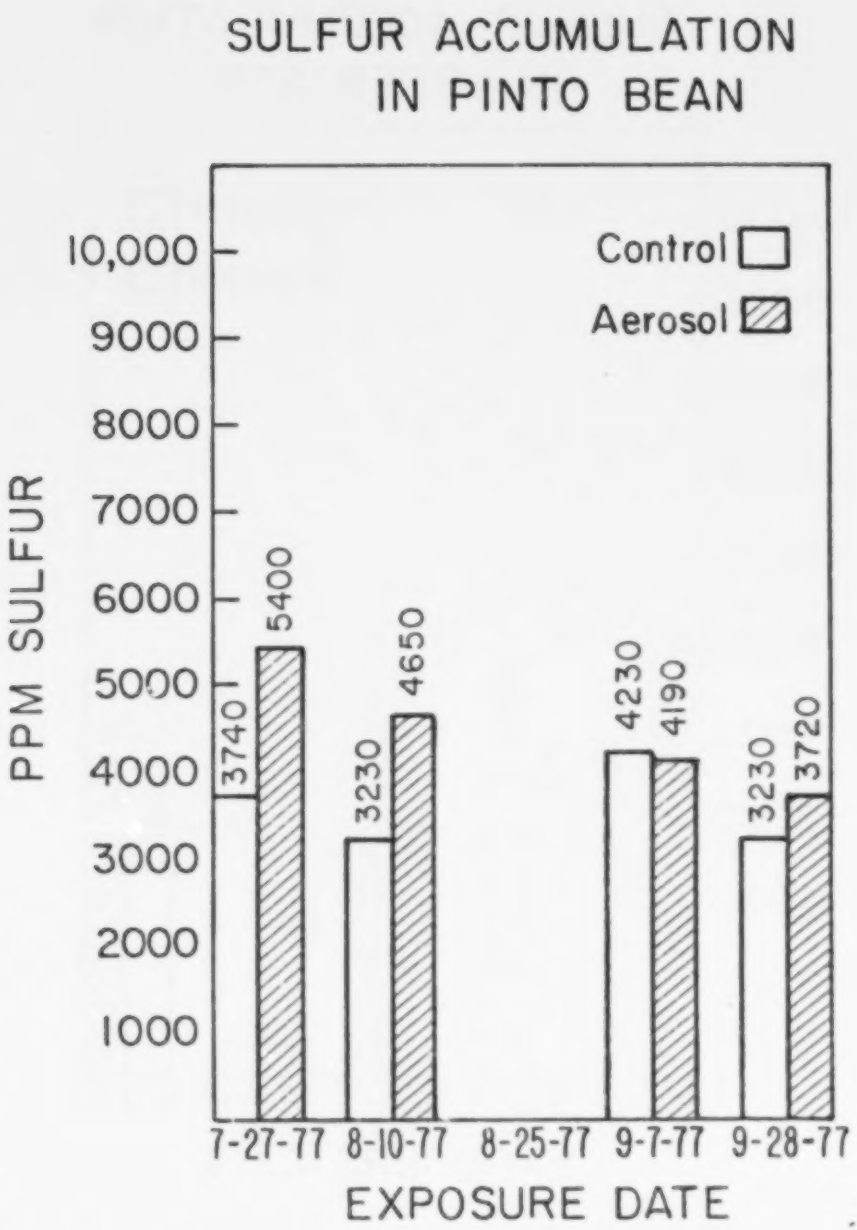


Figure 24. Foliar sulfur accumulation in pinto bean.
Note: season trend showing decreased sulfur accumulation.

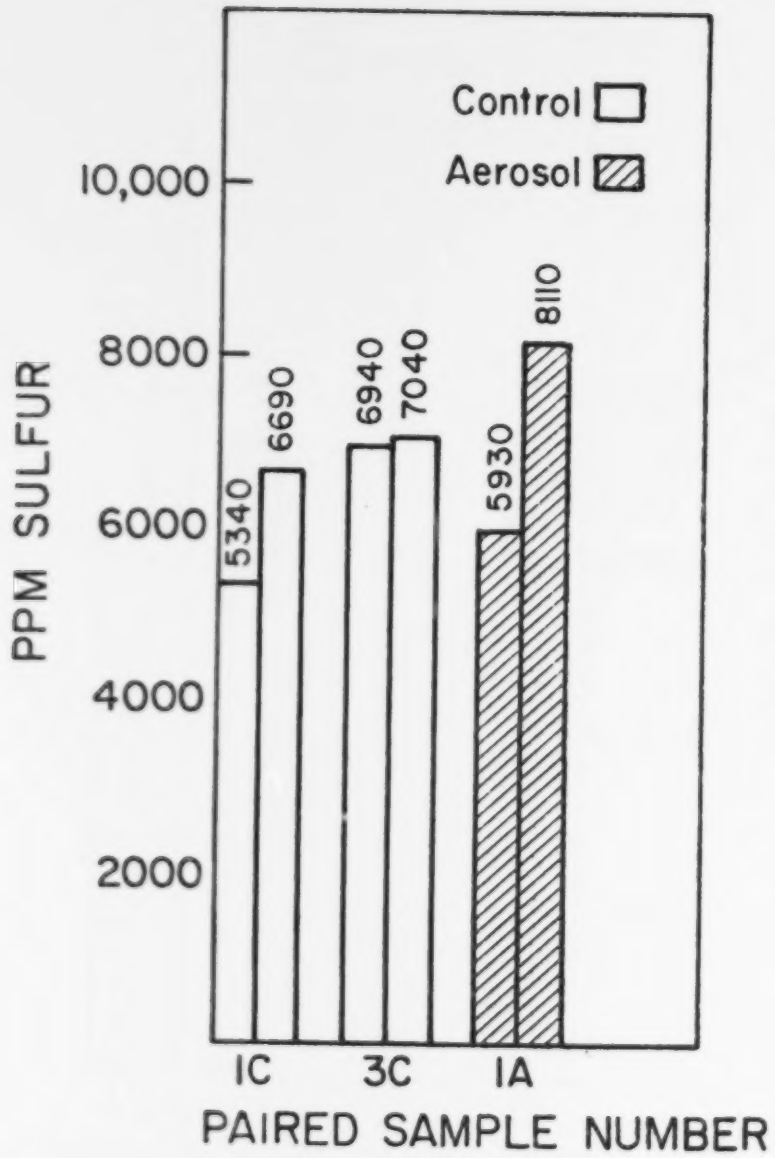


Figure 25. Sulfur accumulation in 7 day old pinto bean plants exposed to 250 mg/m^3 sulfuric acid aerosol for 8 hours.

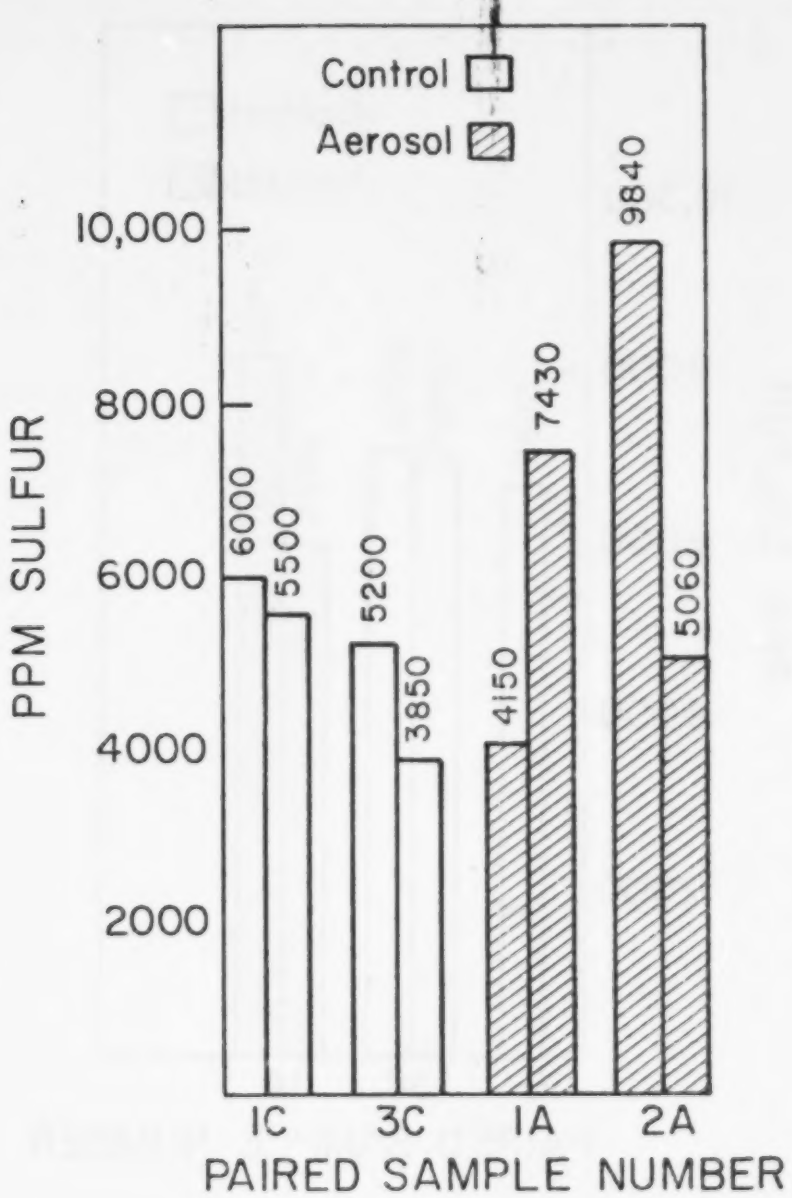


Figure 26. Sulfur accumulation in 9 day old pinto bean plants exposed to 250 mg/m^3 sulfuric acid aerosol for 8 hours when 7 days old and 250 mg/m^3 sulfuric acid aerosol for 8 hours when 9 days old.

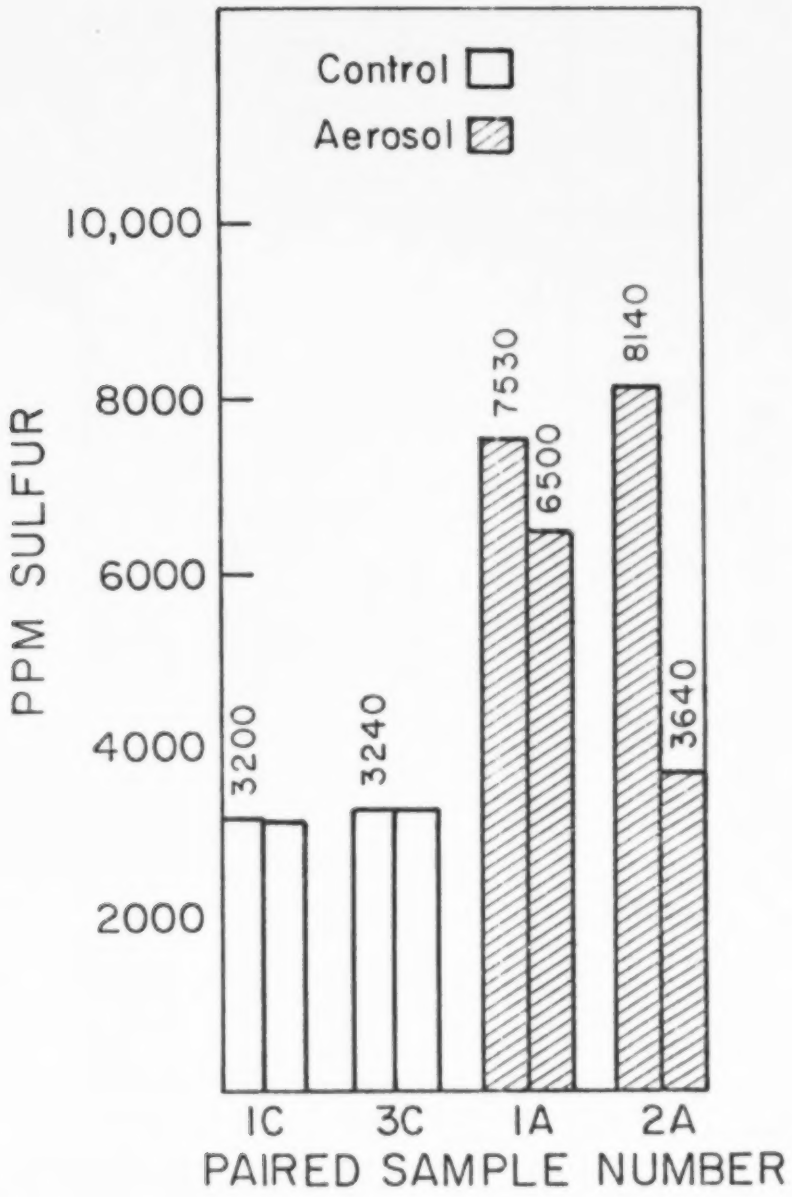


Figure 27. Sulfur accumulation in 11 day old pinto bean plants exposed to 250 mg/m^3 sulfuric acid aerosol for 8 hours when 7, 9 and 11 days old.

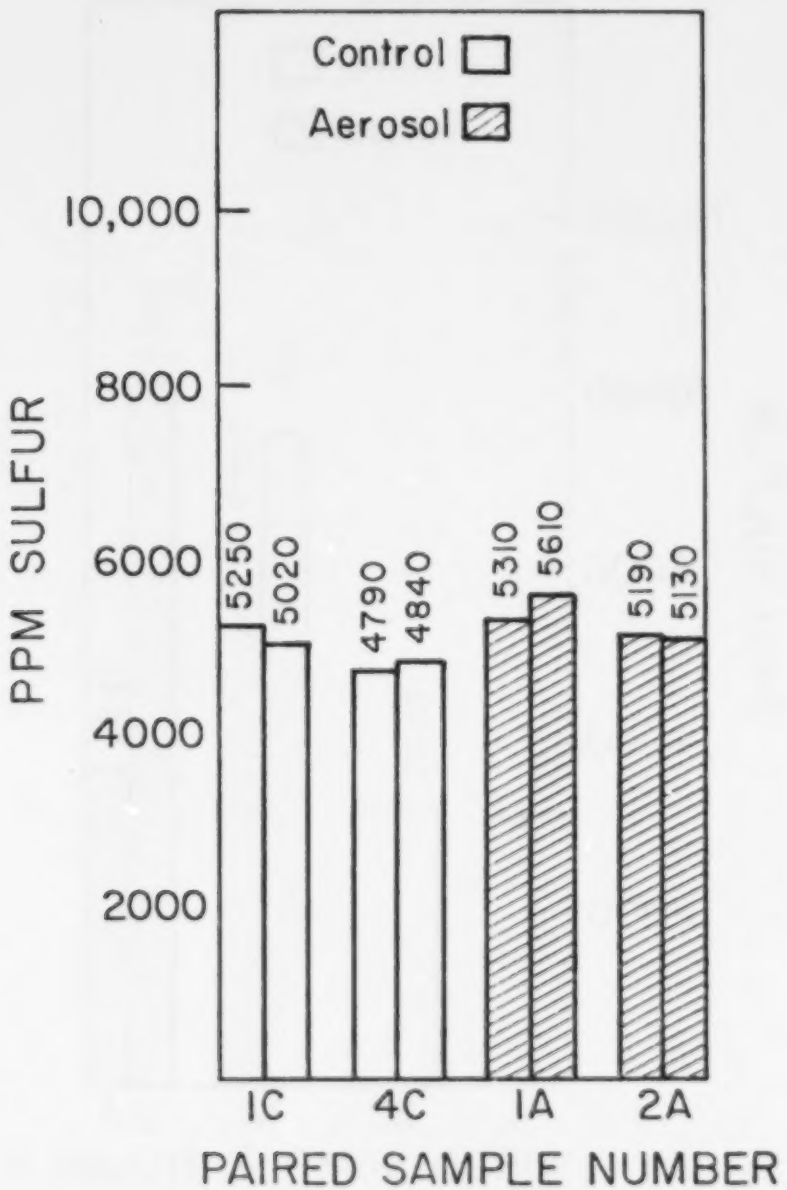


Figure 28. Sulfur accumulation in 16 day old pinto bean plants exposed to 175 mg/m^3 sulfuric acid aerosol for 7 hours.

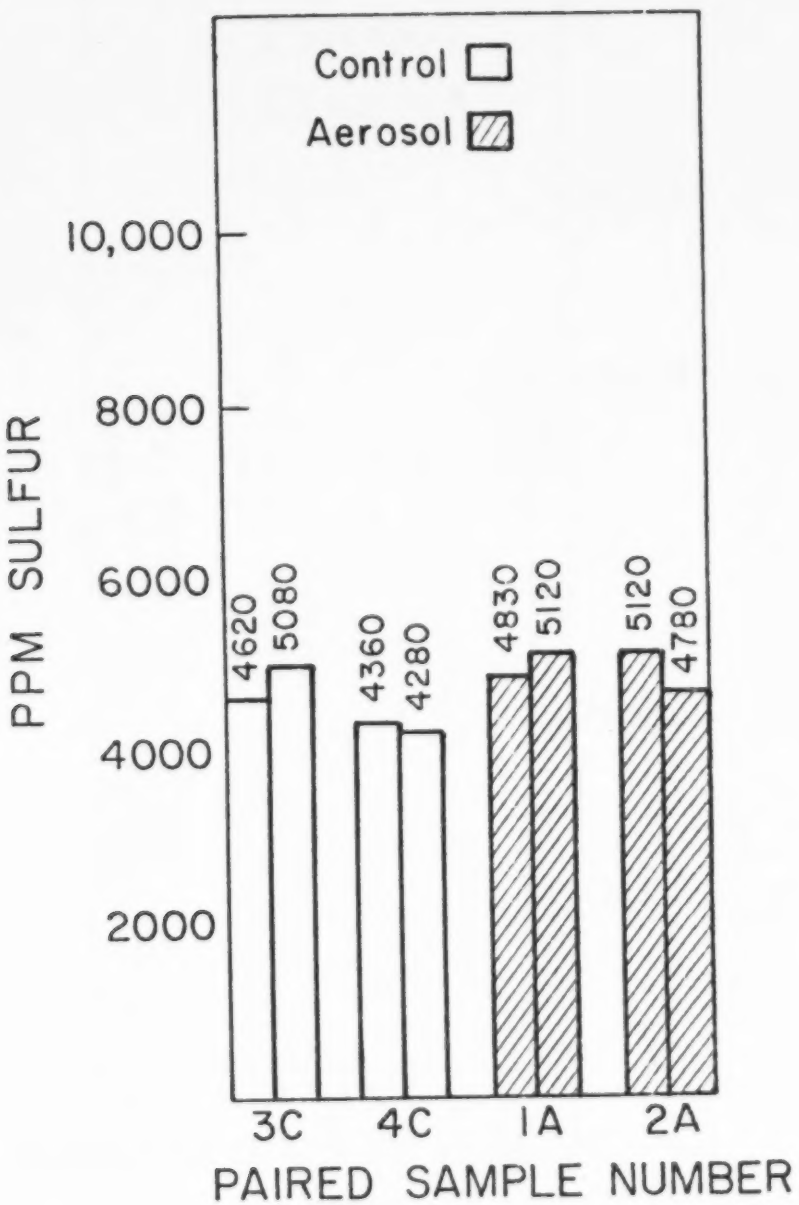


Figure 29. Sulfur accumulation in 26 day old pinto bean plants exposed to 175 mg/m^3 sulfuric acid aerosol for 7 hours.

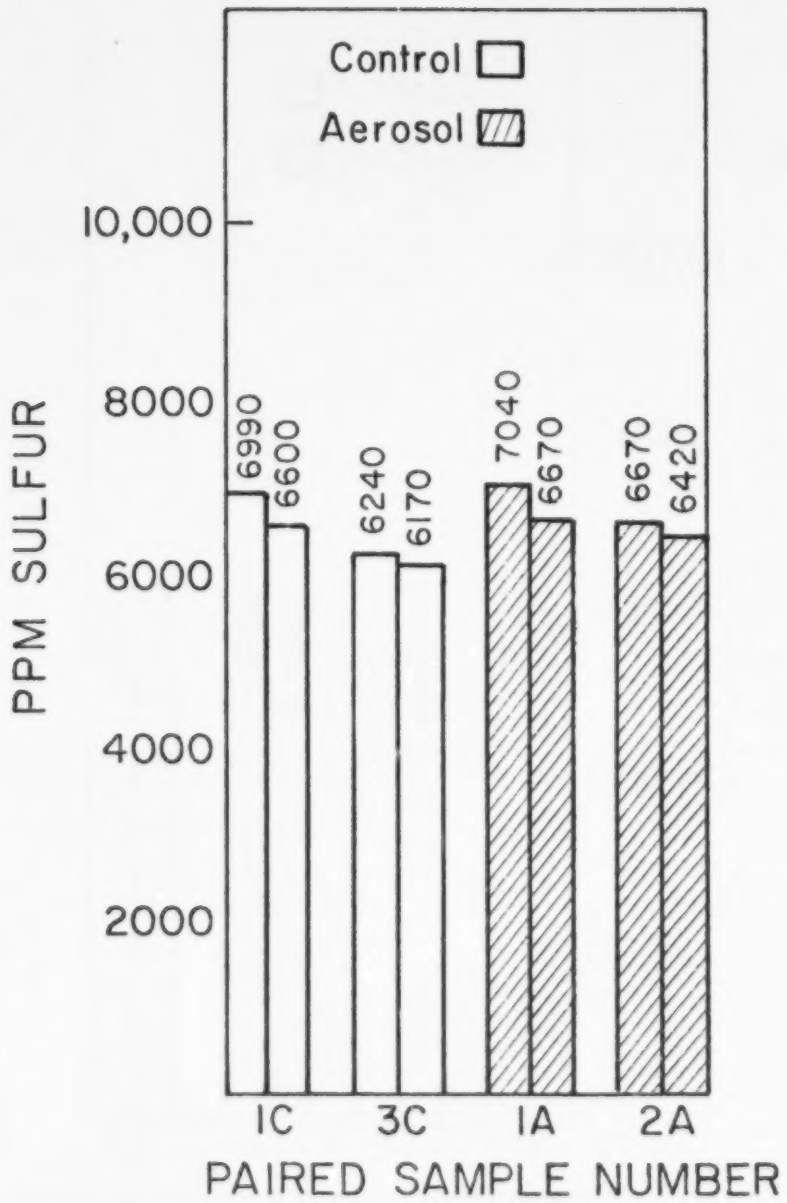


Figure 30. Sulfur accumulation in 19 day old pinto bean plants exposed to 175 mg/m^3 sulfuric acid aerosol for 5 hours.

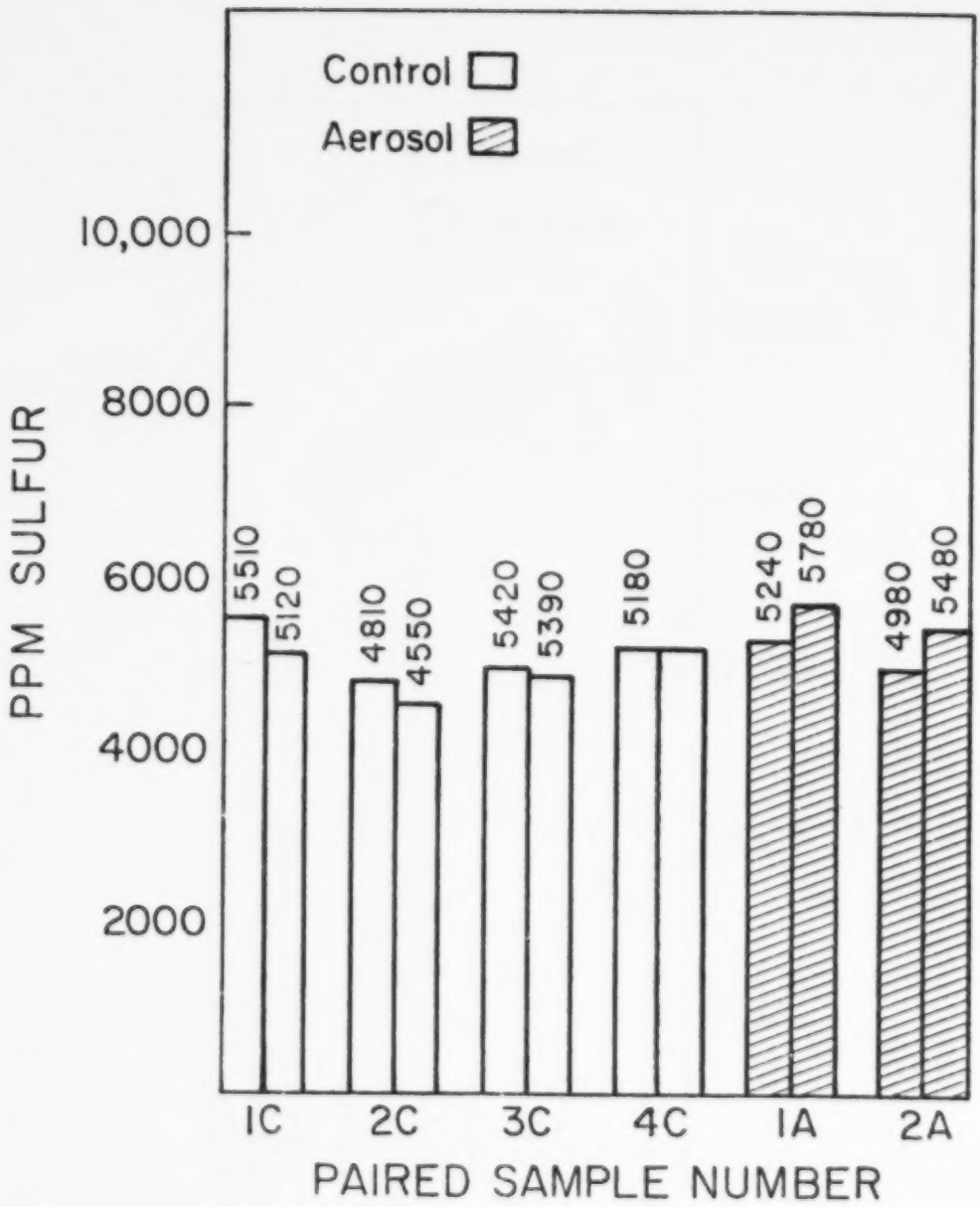


Figure 31. Sulfur accumulation in 43 day old pinto bean plants exposed to 175 mg/m^3 sulfuric acid aerosol for 5 hours.

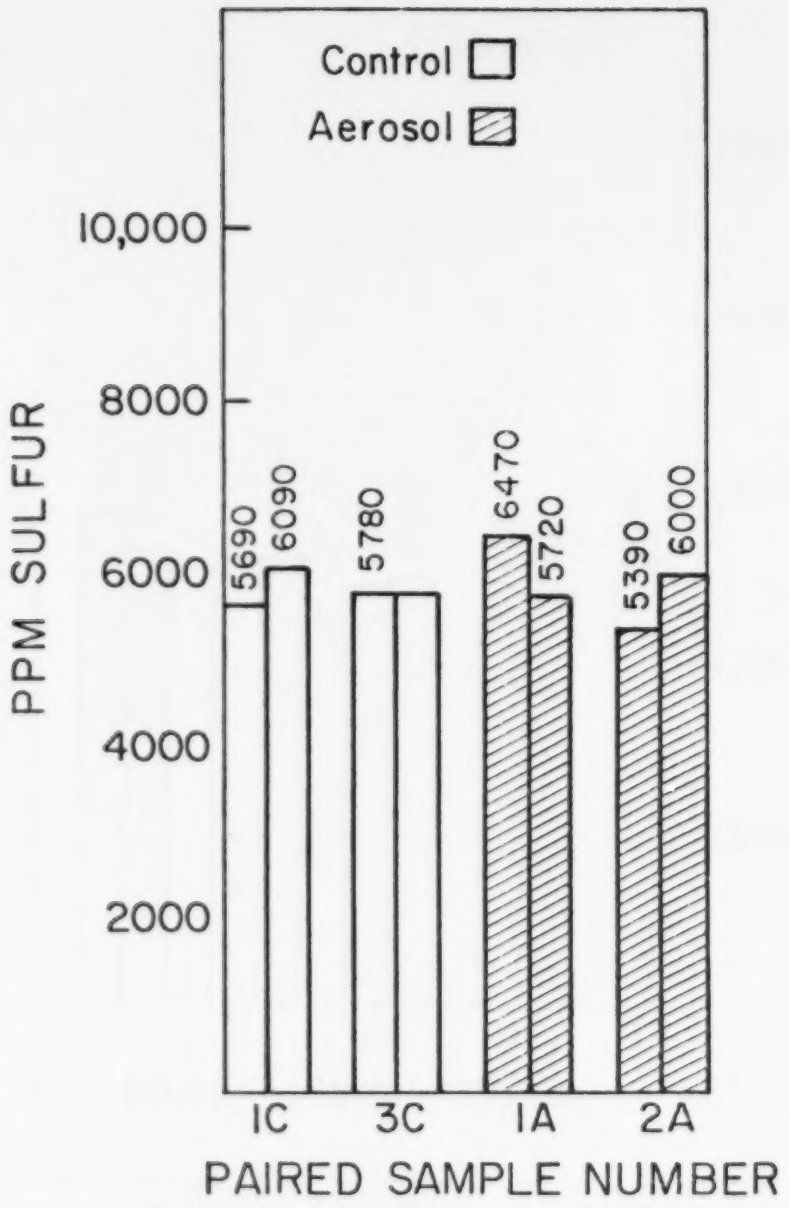


Figure 32. Sulfur accumulation in pinto bean plants exposed to 175 mg/m^3 sulfuric acid aerosol for 8 hours.

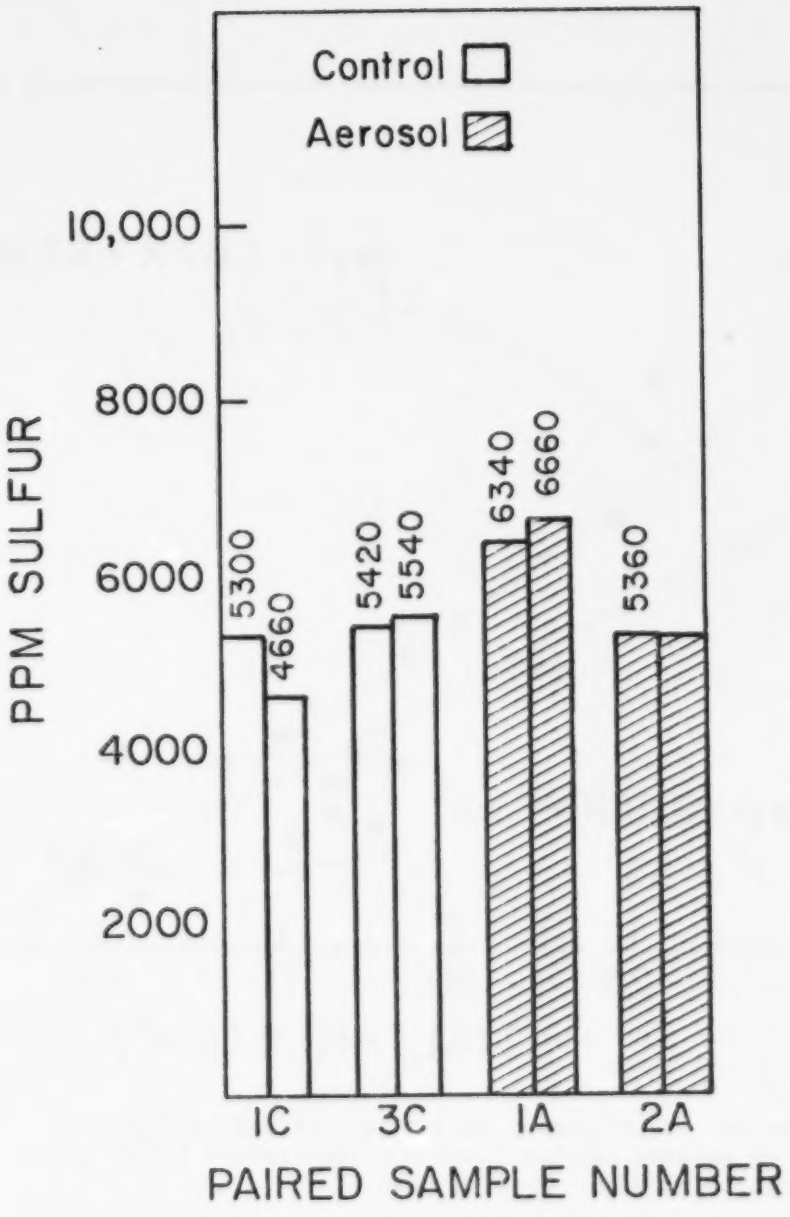


Figure 33. Sulfur accumulation in pinto bean plants exposed to 390 mg/m^3 sulfuric acid aerosol for 12 hours.

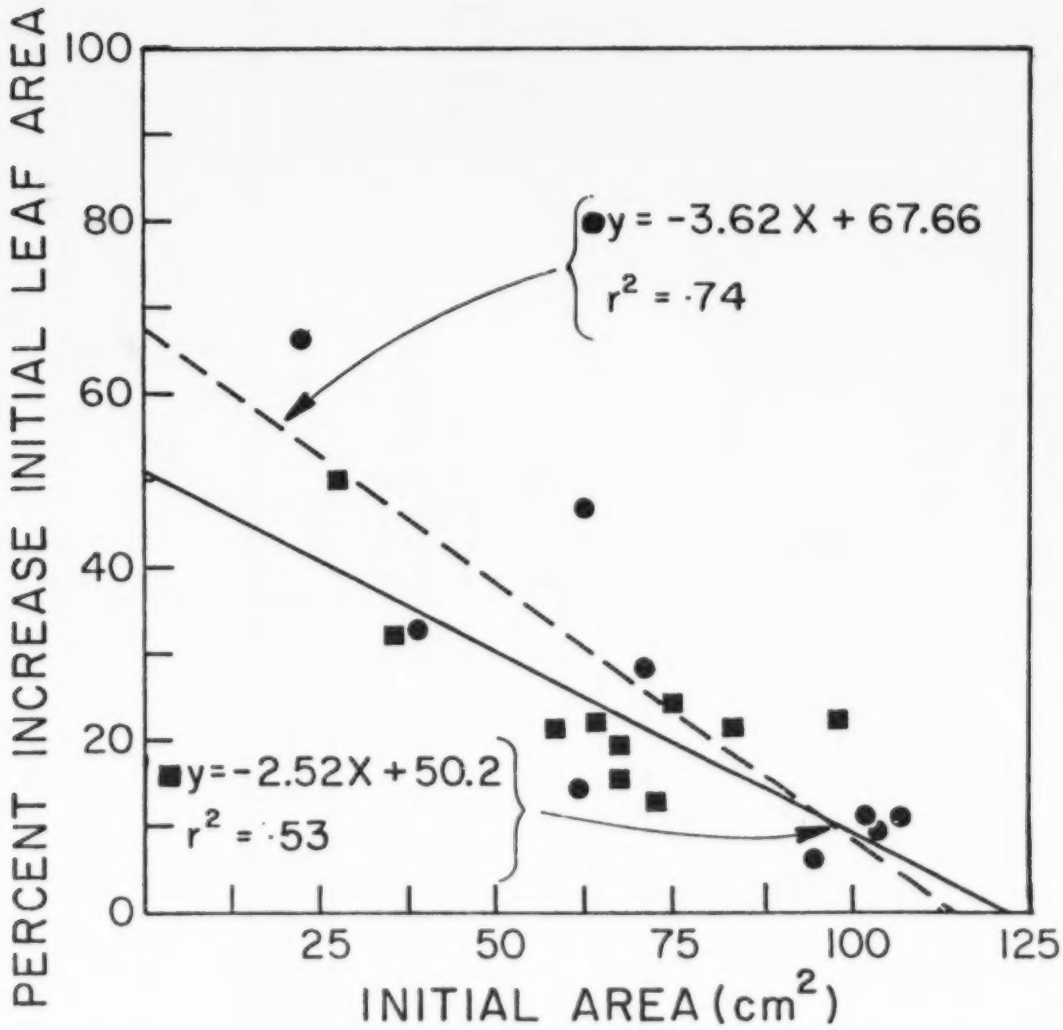


Figure 34. Relationship between growth and initial leaf area of exposed (dashed line) and non-exposed (solid line) trifoliolate leaves of pinto bean.

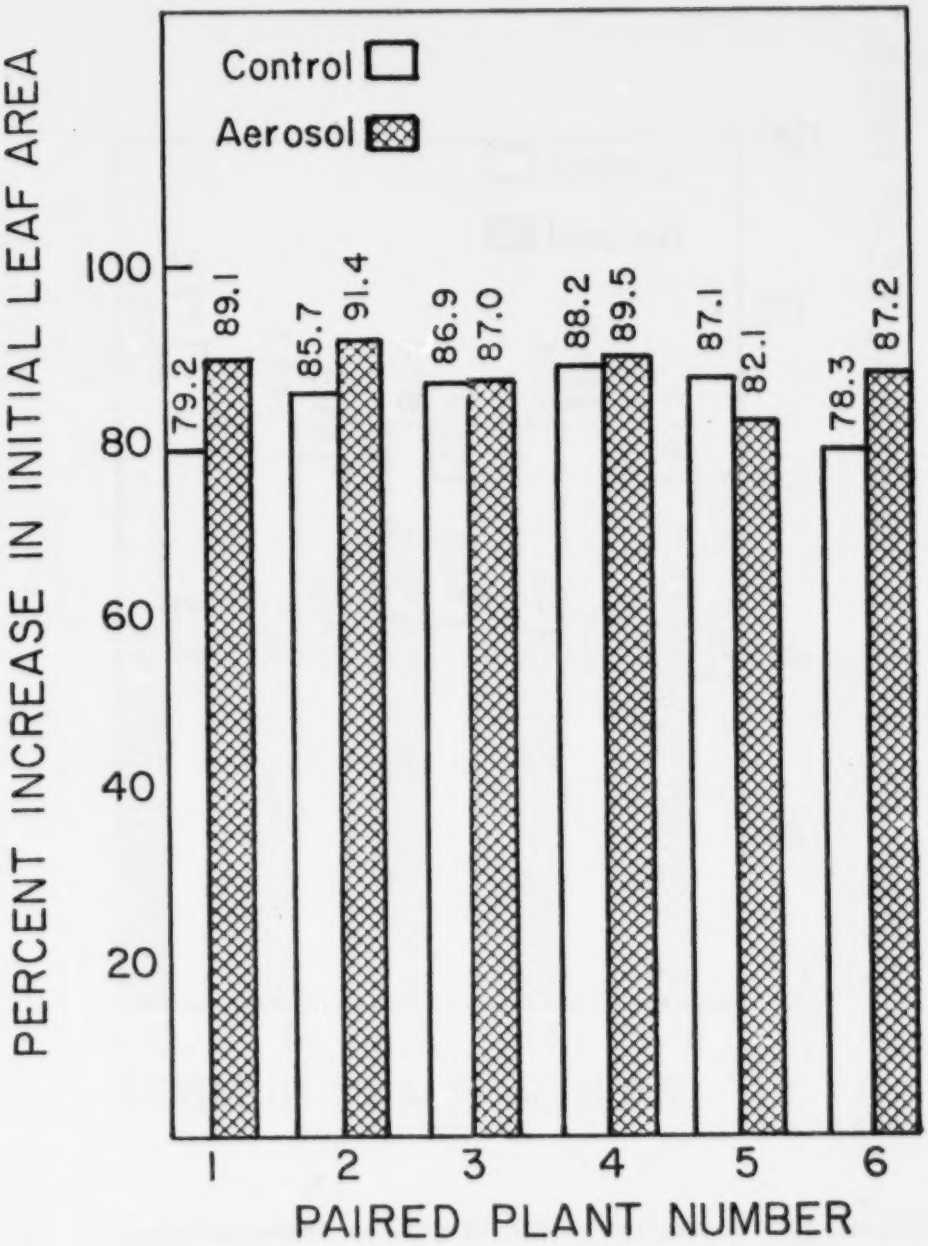


Figure 35. Growth of trifoliolate leaves of pinto bean plants with similar initial leaf areas exposed and not exposed to sulfuric acid aerosol.

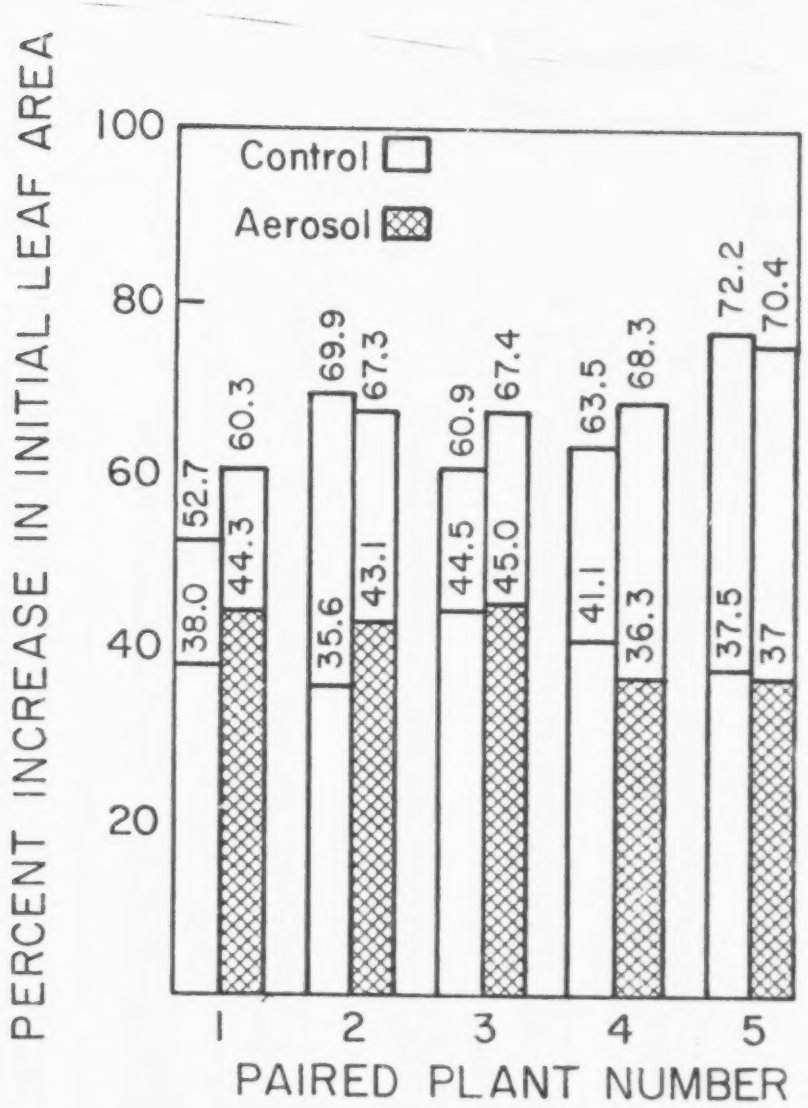


Figure 36. Sequential growth of primary leaves of pinto bean plants at two week intervals exposed and not exposed to sulfuric acid aerosol.

10. EFFECT OF AMMONIA NEUTRALIZATION OF SULFURIC ACID AEROSOL DURING PLANT EXPOSURES

Pinto bean plants were exposed in groups of 25 to ambient air, ammonia alone, sulfuric acid aerosol alone and ammonia plus sulfuric acid aerosol. This experiment was done twice. Anhydrous ammonia was metered to the aerosol chambers in sufficient quantity to neutralize 150 mg/m^3 sulfuric acid aerosol. This concentration of ammonia was much more injurious to vegetation than was 150 mg/m^3 of sulfuric acid aerosol. All plants exposed to ammonia alone wilted and died within 48 hours after exposure. Ammonia combined with sulfuric acid aerosol substantially reduced injury to vegetation. Some injury from ammonia was apparent on foliage when the ammonia concentration was in excess of that required to neutralize the sulfuric acid aerosol in the chamber. Injury was reduced such that no visible symptoms were produced on plants exposed to ammonia with a slight excess of sulfuric acid aerosol. Refinement of the technique for measuring and reacting ammonia and sulfuric acid aerosol is necessary before quantitative information can be gained. These results indicate, however, that sulfuric acid aerosol and ammonia do react to form neutralization products (6) which have less acute effect on vegetation than do either ammonia or sulfuric acid aerosol alone.

DISCUSSION

Acidic substances from the atmosphere have been widely implicated in the production of adverse effects when deposited upon terrestrial ecosystems (8,20,29). Most of the previous studies in this area have related to the effects of acidic precipitation on foliage (8,26). Since a significant part of the acidic material in the atmosphere is now known to occur as aerosol (1), rather than as precipitation droplets, additional studies were needed to assess the effects of aerosols on vegetation. Sulfuric acid aerosol is known to be produced during the combustion of fossil fuels by the oxidation of elemental sulfur and subsequent reaction with ambient water vapor. Automobiles equipped with catalytic converters were recently found to be new sources of this pollutant (11). This project was thus implemented to address the environmental conceras relating to the impact of sulfuric acid aerosol on vegetation.

It was necessary to develop a system for exposing plants to sulfuric acid aerosols, to examine this problem, since previous studies had not been done. The exposure system described here has proven effective in meeting criteria relating to aerosol generation, distribution, deposition, and removal for short term exposures of less than 24 hours. Plants were found to respond to high aerosol concentrations of 100 to 200 mg/m^3 during short exposures of 4-16 hours. The results indicate that acute exposures of this kind to sulfuric acid aerosol are injurious to vegetation and induce both

macroscopic and subtle responses.

It was found necessary to study plant responses under these conditions to produce symptoms characteristic of this form of pollution so that other factors influencing injury could be assessed. Visible injury from sulfuric acid aerosol resembles that caused by gaseous fluoride. Subtle responses which have been observed include foliar sulfur accumulation and the collapse of epidermal and guard cells. Plants are not likely to develop any visible injury from short term (4-16 hour) exposures to sulfuric acid aerosol at concentrations which could be attributed to catalyst equipped cars in the ambient environment (1 $\mu g/m^3$ to 4 $\mu g/m^3$). Concentrations much higher than these (near 100 mg/m^3) are required to produce acute plant responses.

Predicting acute plant responses to sulfuric acid aerosol appears to be complicated by factors under biological control. It was not possible in these studies to accurately define plant responses, either visible or subtle, in terms of physical factors known to condition plant responses to other pollutants. Plants did not show predictable changes in injury related to changes in temperature. Nor was injury increased with increasing pollutant concentration after a threshold concentration was reached. Similarly, preliminary investigations of relative humidity and light influences did not demonstrate predictable relationships.

These observations are not intended to suggest that physical factors are not involved in conditioning plant responses to sulfuric acid aerosol injury. It is probable that optimum temperatures,

concentrations, humidities and lighting regimes exist which will, when the plant is in a susceptible condition, yield maximal injury. However, highly variable observations relating to plant responses at different times to a constant level of a given physical factor has led us to speculate that biological factors may be principally involved. Several authors have provided rationale appropriate to this conclusion.

Sulfate uptake and metabolism have been reviewed by Schiff and Hodson (25). These authors have discussed several factors which we did not resolve in this study. Of the major elements required by organisms, sulfur is unusual in that it can be utilized as sulfate, its most highly oxidized natural form. Most plants and animals are capable of oxidizing reduced sulfur compounds to sulfate but sulfate reduction reactions are primarily restricted to the plant kingdom. Sulfate reduction is required during synthesis of many sulfur containing amino acids and proteins. Organisms which can reduce sulfate are capable of using sulfate as a sole source of sulfur. The reduction process is expensive in terms of energy, requiring about 180 kcal/mole for reduction to the thiol level (25). For this reason, highly evolved species may have lost this ability (25).

The ability of plants to metabolize sulfate brings into consideration a number of factors which may complicate and interfere with aerosol exposure studies. Varied plant responses to sulfate as described in the results section may be a reflection of their effects. Sulfate uptake seems to be accomplished by active transport mediated by a carrier with enzyme properties (25,27). Transport is unidirectional

from outside to inside the cell and is governed by pH, temperature, sulfate concentration and available metabolic energy. Free internal sulfate is also known to depress sulfate transport as is excess endogenous sulfur, especially as cysteine and methionine (25,27).

Possibly the growth stimulation shown in Figure 34 for young leaves exposed to sulfuric acid aerosol was due to the reflief (21) of an initial endogenous sulfur deficit. Conversely, the growth inhibition of older leaves may have been due to a net excess of endogenous sulfur induced by sulfate accumulation . The foregoing considerations make it apparent that plant responses to acute sulfate exposure may be unpredictable, unless an intensive physiologic study is done concurrently.

Plant response to sulfuric acid aerosol is further regulated by stomates. The basic mechanism of stomatal action is not yet understood (10,22). What is know indicates that this action is also complicated and may be unpredictable in many environments (2,14). It is currently thought that stomates respond first to interstomatal carbon dioxide concentration and only indirectly to other factors such as light which subsequently influence carbon dioxide flux (22). Stomatal action is known to be energy dependent. Each opening requires at least 0.5 erg and is consequently controlled by uncoupling agents such as dinitrophenol and inhibitors of energy supply or high energy phosphate transport such as sodium fluoride (22). Since sulfate activation to adenosine 3'-phosphate 5'-phosphosulfate (25) is thought to be necessary prior to transport it is possible that the same factors which control stomate closure and opening may prevent sulfate transport. Simultaneous interference with these processes would be likely to cause substantial

changes in plant response to sulfuric acid aerosol exposure.

Raschke has proposed a model for stomatal action based upon guard cell turgor pressure controlled by metabolism of organic acids and transport of inorganic ions (principally K^+, H^+ and Cl^-). Malate is thought to be the primary organic acid involved in this process. The direction and magnitude of transport is dependent upon the pH of the cytoplasm which in turn controls the concentration of malate. Carbon dioxide serves as the substrate for acidity and the basis for osmotica production. Other acids such as abscisic acid are known to enhance the effect of carbon dioxide and act to expel H^+ from the guard cells. It seems probable that exposure to and uptake of other acidic species could play a similar role and act to close stomates. These processes are known to be actively controlled as was previously described.

A consideration of the complex interactions regulating sulfate uptake and stomatal action can help explain the varied responses described in the results section. Plants were found to be alternately sensitive and insensitive to conventional factors controlling injury. Pollutant concentration, temperature, duration of exposure and age of plants did not appear to be fundamentally involved in determining plant responses to sulfuric acid aerosols. Based on the foregoing discussion, it is suggested that several additional factors may be involved in conditioning plant response to sulfuric acid aerosol. These include endogenous plant sulfur (especially as sulfate), plant energy status, interstomatal carbon dioxide concentration, and stomatal diffusion resistance. Quantification of these factors, in conjunction with aerosol exposure, will require refinement of state of the art techniques

in plant physiology for use during plant exposures. Application of these techniques will be necessary to understand the observed seasonal trends and will be essential to the development of reproducible studies involving chronic exposures and subtle effects.

Most of the work reported here has involved the acute exposure of plants to fine aerosols of sulfuric acid. Most plants were exposed to sulfuric acid aerosol concentrations in the range of 100 to 200 mg/m^3. Exposures were for short times of less than 24 hours. These conditions were not intended to be analogous to those found in the ambient environment. They were designed to produce acute plant responses (necrosis and wilt) which could then be related to subtle plant responses (pollutant accumulation and growth). Correlation of acute and subtle plant response to pollution is necessary to predict when significant injury will occur from chronic exposures. Whitby (34 has reported atmospheric sulfate concentrations of 80 $\mu g/m^3$ under highly polluted conditions. This value is two to three orders of magnitude less than the concentration we have found necessary to induce acute injury to vegetation and is 10 to 100 times larger than that which could be attributed to automobiles equipped with catalytic converters. Additionally, the relative effects of hydrogen and sulfate ions deposited as acid or neutralized salt aerosol (12) were not evaluated. It is likely that under most ambient conditions plants will be exposed to mixtures of pollutants including aerosols, oxidants and oxides of sulfur and nitrogen. Investigations of these multivariable interactions are needed.

There is presently a need to extend these results to include chronic

plant responses to sulfuric acid aerosol at concentrations which occur in the environment. Chronic exposure studies will require increased stability of the exposure system and are additionally complicated by the observation that injury appears to be conditioned by biological as well as physical factors.

The exposure system should be modified somewhat for continuous operation during chronic exposure studies. The compressed air cylinders, while providing an excellent degree of air contaminant control, could be replaced with a compressor system fitted with appropriate pollutant filters and air dryers. Additionally, the problem of changing cylinders during long term exposures would be eliminated. The aerosol generator equipped with the Teflon nebulizer modification and perhaps a Teflon impaction stage should provide good service during continuous operation.

The principal problem associated with long term plant exposures relates to the changing responses of growing plants and presents a paradox of sorts. The objective addressed in designing this system was to develop a reasonably closed exposure system in which physical variables could be controlled. This becomes increasingly difficult as the duration of the exposure is increased. It may also be anticipated that more changes in the biological variables which condition response to pollutant stress will occur in proportion to the length of time the plants are in an exposure chamber. This increased variability in plant response is superimposed upon a decrease in degree of response at lower pollutant concentrations. The researcher is thus confronted with a paradox: more variability in frequency of response and a decrease in the intensity of the response which is to be measured. These aspects of

chronic plant exposure call for an additional degree of refinement in the exposure system. Refinement may be accomplished in part, by operating the system as a reactor in which incoming and outgoing pollutant concentrations are known, and uptake can be thereby predicted. Such a system has been described by Hugo Rogers (PhD Thesis North Carolina State University 1975).

Much work remains to be done to resolve these questions. It is apparent that sulfate loading of the atmosphere from fossil fuel combustion with subsequent deposition on terrestrial ecosystems will continue into the predictable future (9). The ultimate fate and effect of sulfate on ecosystems is a question is demand of an answer. If plants are found to accumulate sulfate in excess of their ability to utilize sulfate, a decline in the productivity of photosynthetic systems may be anticipated with significant subsequent effects on the health and welfare of mankind.

SUMMARY

1. A continuous flow system for exposing vegetation to submicron sulfuric acid aerosols has been designed and constructed. All surfaces within the exposure system which are in contact with sulfuric acid aerosols are composed of either Teflon or stainless steel and are essentially inert to sulfuric acid corrosion.

2. Exposure chambers were designed to allow simultaneous exposure of different branches of the same plant to aerosol and control environments to minimize variation associated with individual plant differences.

3. Submicron sulfuric acid aerosols are mechanically produced using a nebulization-impaction-neutralization process. Typical aerosol distributions have a mass mean diameter of about 0.5 μm and a standard geometric deviation near 1.7.

4. Plants are injured during acute exposures to sulfuric acid aerosols. However, the aerosol concentrations required to produce visible injury are several orders of magnitude higher than those which have been attributed to sulfate in the ambient environment.

5. All plant species exposed to sulfuric acid aerosols have shown the same characteristic injury to leaf margins and tips.

6. This is the first report of this form of injury attributable to acidic pollutants.

7. Plants vary in susceptibility to sulfuric acid aerosol injury.

8. Plant responses to sulfuric acid aerosol have not followed proportional relations to pollutant dose (concentration X time).

9. Lack of proportional dosage relations suggests that active rather than passive mechanisms may be controlling plant response to this pollutant.

10. Simultaneous exposure of plants to ammonia and sulfuric acid aerosol in stoichiometric quantities appears to decrease visible injury normally associated with exposure to sulfuric acid aerosol alone.

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